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Fire as a selective agent for both serotiny and nonserotiny over space and time

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Running head: Fire and serotiny

Abstract. Serotiny is the prolonged storage of seeds in closed supporting structures retained within the plant crown. The list of known serotinous species in fireprone regions of Australia, South Africa, Mediterranean Basin, North America and Asia now stands at 1345, many of which dominate their ecosystems. Length of storage varies from a few years (weak serotiny) to more than 10 years (strong serotiny). Serotiny is the storage part of a reproductive syndrome that includes rapid seed release in response to an environmental cue, usually fire. Strong serotiny is promoted when recruitment of postfire seedlings is favoured over interfire seedlings with the net effect that the 'gene support for serotiny' builds up rapidly in successive generations. Nonserotiny is favored when interfire seedlings are more likely to survive either because the habitat is non-fireprone or because fire may occur annually but adult plants survive. Propensity for serotiny shows remarkable trait diversity within clades, some covering the full range of possible fire-regimes (*Pinus*, *Protea*, *Banksia*, *Hakea*). Co-occurring congenerics may possess contrasting levels of serotiny under moderately frequent, intense fires. We list 23 traits associated with the serotiny/nonserotiny syndromes that are subject to both environmental and phylogenetic constraints; all are coordinated for maximum fitness but a trade-off between serotiny and resprouting is not apparent. Some traits are only indirectly related to selection by fire, such as protection from granivores, that may interact with the fire regime. Both serotiny and nonserotiny have a long history among woody plants that can be traced from the Triassic, escalating in the Cenozoic and peaking over the last 5 million years. A marked drop in serotinous-lineage proliferation occurred in the cold 40–30-million-year period. Some species in all clades have returned to the nonserotinous state of their ancestors as fire frequency has either increased markedly or they migrated to fire-free habitats. Progress on determining the heritability of serotiny could be enhanced by using temporally based measures of serotiny. The (epi)genetic/morphological/physiological basis for early or late release of seeds from cones/fruits remains unknown. Anthropogenic influences are having a profound effect on the conservation status and evolutionary trajectory of serotinous species.

Key words: Banksia, canopy seed storage, fire ecology, flammability, Hakea, interfire, mediterranean, Pinus, postfire recruitment, Protea, resprouters, savanna, seedlings, seed storage, woody fruits

SEROTINY *VS* NONSEROTINY

Serotiny refers to prolonged on-plant storage of seeds and is part of a major reproductive syndrome among woody plants (Box 1) that can be distinguished from seed release at maturity (nonserotiny) and soil storage (geospory). The ecology and evolution of serotiny has been widely studied in North America, Mediterranean Basin, Australia and South Africa where it is recognized as a key adaptive trait to fire (Lamont and Enright, 2000; Keeley et al., 2011; Treunicht et al., 2016) with an evolutionary history that extends back to the Triassic (He et al., 2016a; Lamont, He and Yan, 2019a). The adaptive advantages of serotiny among woody plants center on its ability to increase seed availability above the minimum required for postfire population recovery (Enright et al., 1998a). The relative benefits of different levels of serotiny revolve around the opportunities for interfire recruitment compared with postfire recruitment (Cowling and Lamont, 1985a; Enright et al., 1998a). Adaptive changes in the level of serotiny are expected when a) a species attempts to invade an area whose fire regime is different from the current one, or b) the fire regime changes in its native habitat

(Ne'eman, Goubitz and Nathan, 2004; Lamont, He and Downes, 2013). Thus, nonserotiny can be expected in non-fireprone habitats where postfire germination is not an option (Lamont, He and Yan, 2019b). Less well-known is the fact that high fire frequency may also induce nonserotiny, or more generally, nonstorage (Buma et al., 2013; Lamont, He and Pausas, 2017a). When fire is likely in most years, as in (sub)tropical savannas, there is little opportunity for, nor benefit in, the accumulation of seeds, and, in fact, flames may not reach the crown of trees anyway. Thus, the extremes of fire frequency both promote nonserotiny, and the processes responsible are discussed here.

The purpose of this review is to explore the environmental factors associated with fire (or its absence) that promote or inhibit the evolution of serotiny spatially (different fire regimes and growing conditions), at different levels of organization (within and between populations, species, genera and broader clades), and at different temporal scales [currently (microevolution) and over geological time (macroevolution)]. We begin by describing the serotinous structures that protect their stored seeds and their taxonomic and geographic distribution. Consideration of the syndrome of traits associated with on-plant seed storage, seed release and seedling recruitment follows. What is the genetic basis for these fire-adapted traits? The relevant genes and alleles arise from somatic or meiotic mutations (Benca, Duijnstee and Looy, 2018; He and Lamont, 2018) and we assume that they already exist at low frequencies among some genotypes. Prior adaptive responses to other survival hazards, such as herbivory, seasonal drought or other disturbances/stresses (in this review, x/y means "x and/or y") may play some role but fire usually precedes them as a selective agent (Lamont, He and Yan, 2019b). We concentrate on the selection process because little is known about the initiation of genetic mutations while the role of fire as a selective agent is well established (Castellanos et al., 2015). Next considered are impact of the population dynamics of interfire and postfire recruitment and phenotypic selection, followed by the possible genetic mechanisms that increase or decrease the gene 'support' for serotinous traits. We then review actual examples of the outcome of the selection processes described above, first at the intraspecific scale and then at the interspecific, intrageneric and intracladal scales. These highlight different responses between populations and taxa to different constraints, and different responses to the same constraints by different taxa. We then document changes in the occurrence of serotiny and nonserotiny through geological time. Issues for conservation and priorities for future research are noted at the end.

MORPHOLOGY, TAXONOMY AND DISTRIBUTION OF SEROTINOUS STRUCTURES (Figs. 1, 2)

There is a great diversity of serotinous structures (Fig. 1) among the 1345 species, distributed across 55 genera in 12 families (Fig. 2), documented in this review. All seed-bearing structures, but two types with fleshy sepals, are dry and variously woody, and all are dehiscent at the level of the cone (that releases seeds or indehiscent fruits) or fruit (that releases seeds) (Table S1). The data for species yet to be examined indicate that the total listed here is a little more than half of the likely total number (~2500). Seven genera are currently recorded with ≥ 50 serotinous species (*Eucalyptu*s to *Callistemon* in Fig. 2). Within serotinous genera, 21 (38%) also contain nonserotinous species, especially among the largest genera, while 53% are solely serotinous. Regionally, 87.5% of records are for Australia (taking into account estimated underestimates of the numbers in Eastern Australia), with 75% of these in SW Australia (that occupies 4% area of the continent). South Africa accounts for 10.5% of species, with 1.5% in North America and 0.5% in the Mediterranean Basin, and two species in Asia (*Pinus*

yunnanensis, *Larix gemilinii*). Regarding serotinous structures, 43% are isolated or loosely clustered fruits (terminal), 30% are cones (terminal) with indehiscent fruits, 25% are aggregated fruits (axillary, except for *Syncarpia*) and 4% are cones with seeds (terminal), all pictured in Fig. 1 and described in Table S1. Homology exists within the fruit type (follicle, capsule, achene) and within conflorescence type (solitary, umbel, capitulum, cyme) but not between them (homoplasy). Pinaceae, Casuarinaceae and the two major subfamilies of Proteaceae (Proteoideae, *Banksia* in Grevilleoideae) have quite different cone types structurally. The first stores naked seeds supported by fused scales (bracteoles), the second has fruits immersed between two woody scales, the Proteoideae has one woody scale pressing the fruit against the rachis, whereas *Banksia* has protruding fruits immersed in a mantle of fibrous bracts per two florets each with its own fibrous bracteole (George 1981). As for the dispersal units, 70% are air buoyant with 51% having winged seeds, 19% with winged, hairy or otherwise buoyant fruits, 29% with small (< 2 mm long), wingless seeds, and 1.5% with wingless fruits (*Leucadendron*). All these structures appear to have evolved independently although subjected to the same forces of selection (discussed later).

TRAITS THAT CONTROL SEROTINY, SEED RELEASE AND RECRUITMENT (Fig. 3, Table 1)

If we are to consider fire as a selective agent for the evolution of serotiny/pyriscence or their reverse, then we need to know on what traits fire must act for adaptive change to occur. The first three of six stages are common to plants in general (Fig. 3). Species possess a syndrome of traits that are adapted to three constraint dimensions – the prevailing abiotic environment (climate, soils, topography), the prevailing biotic environment (pollinators, herbivores, granivores, pathogens, dispersers) and disturbance, principally fire (Pausas and Lamont, 2018; Lamont, He and Yan, 2019b). Thus, the pre-existence of adaptations (genetically based traits selected for over time) to the abiotic and biotic environments and fire regime occupied are prerequisites. Serotiny (*vs* nonserotiny) are the initial components of two reproductive syndromes that consist of a) build up of an aerial seed bank (*vs* release at maturity), b) release of seeds in response to fire heat (*vs* release in the absence of fire), and c) germination and recruitment of seedlings *en masse* postfire (*vs* continuous recruitment in the absence of fire) (Fig. 3). Analysis of one component without reference to the other two will underplay the ecological significance of serotiny. Thus, prolonged storage traits are promoted in an ultimate sense because a) annual seed production is inadequate for self-replacement should a fire occur in that year, b) optimal recruitment conditions only exist immediately post release as a result of fire, and c) seeds released interfire are no longer available to contribute to the next generation (Lamont et al., 1991; Enright et al., 1998a,b). On-plant storage also gives extra time for seed mass and nutrient content to build up, of particular value for seedling recruitment in nutrient-impoverished, seasonally dry soils where the incidence of serotiny reaches its peak abundance (Lamont and Groom, 2013) so is related to point (a) above. This solution to the need for nutrient-enriched seeds would still not favor selection for serotiny were it not tied to fire-stimulated seed release under superior conditions for recruitment afforded by the postfire habitat.

We now discuss 23 traits identified from the literature and associated with these three components that are under genetic control and environmental selection, tempered by phylogenetic constraints (Table 1). Several processes are related directly to storage: propensity to produce enclosure sealants, a vascular system that prevents premature drying out of the storage tissues, and seed viability

that matches the length of storage. Equally, for non-storage, an efficient abscission mechanism is required that may involve rapid occlusion of the xylem once maturity is reached (Moya et al. 2008). Effective serotiny entails a) protection of seeds from the 'elements' over some years in the absence of fire and b) corresponding survival of the protected seeds. However, the supporting structures invariably stay closed for longer than their seeds remain viable. Thus, 85% of 10–12-year-old follicles and seeds of *Banksia cuneata* remain intact, but seed viability is < 35% compared with 85% for one-y-old cones (Lamont, Connell and Bergl, 1991). Death of embryos can be due to postzygotic-acting deleterious alleles, consumption by insect larvae, granivorous birds or rodents, the action of pathogenic fungi or bacteria, desiccation, waterlogging or gradual senescence. Some pine cones have tough, spiny apophyses apparently directed at squirrels or corvids (Elliott, 1974; Benkman et al., 2003; Siepielski and Benkman, 2004); some banksias have a dense mantle of persistent florets that not only increase flammability and ensure seed release (Enright and Lamont, 1989) but also serve to conceal the fruits from granivores; some hakeas have elaborate woody rims over the thinnest, weakest part of the fruit (suture line) deterring granivores, especially cockatoos and ovipositing insects, from reaching the seeds (Midgley, Cowling and Lamont, 1991; Groom and Lamont, 2015; Fig. 3). All these protective mechanisms will have different genetic bases that vary greatly between different plant taxa.

As seed release is essentially a desiccation phenomenon, any drying out of the seed-supporting structures can lead to seed release (dos Santos et al., 2015). Only fire heat causes *en masse* release at the population scale, known as pyriscence (Lamont, 1991). Interfire dehiscence can be the result of plant death or local necrosis of the supporting branches or even of individual fruits or cones (necriscence), usually through failure of the vascular system to maintain water supply to the maternal tissues (Cramer and Midgley, 2009). Direct insolation (soliscence), hot weather and dry air (desiscence – after desiccation, Lamont 1991, Nathan et al., 1999) can also lead to weakening of the binding resins and desiccation, while these effects can occur simply with the passage of time. Weakly serotinous cones/fruits open at lower temperatures than strongly serotinous structures (Enright and Lamont, 1989; Huss et al., 2018) so that they are more likely to release their seeds between fires.

That serotiny is usually terminated by fire heat has implications for the selection of a number of traits (Table 1). Paramount is insulation of seeds from fire heat that requires the genetic propensity to synthesise non-flammable, woody/corky tissues. As it turns out, many species are 'overadapted' for fire resistance as protection from granivores requires even thicker and denser tissues. Further, seeds of many species have high heat tolerances (up to 170°C for 2 min), equivalent to those of soil-stored, 'hard' seeds, while the germination of some others is optimized by temperature pretreatments not expected in the absence of fire (60–80°C), showing that fire has acted as a selective agent even at the pre-release stage. Nevertheless, old, partly open cones (Lamont and Barker, 1988) or dry cones/fruits on dead plants (Lamont and Groom, 1998) are more likely to be incinerated even where viable seeds remain stored on dead branches or plants. Thus, some traits are directed at storage as an end in itself unrelated to the inevitability of fire events, some protect against heat as well as granivores (multifunctional), while others control the heat-induced, seed-release mechanism. All of these will be under separate genetic control.

The cueing of seed release by fire involves a complex mechanism, which must be controlled by numerous genes and alleles, that may include a) the presence and melting of binding resins at the suture line, then b) the presence of specialized tissues that cause reflexing of the enclosures as they

desiccate, sometimes aided by wet-dry cycles. The mechanism is particularly elaborate among banksias and dryandras where a winged central plate gradually pulls the two seeds out of the follicle – it seems that this 'separator' also serves to deter granivorous birds, which descend on the cones as soon as the fire passes, from accessing the seeds (Cowling and Lamont, 1987). If this is the result of selection then its current morphology must be relatively recent as cockatoos only arose ~20 Ma whereas this clade emerged > 60 Ma (He, Lamont and Downes, 2011). At about the same time, other traits arose that enhance the flammability of the region around the cones/fruits and ensure the critical temperature for resin melting is reached – retention of dead florets, leaves and twigs. Serotiny in pines is tied to retention of dead branches that enable propagation of flames from the ground to the crown (the 'ladder' effect) where the closed cones are located (Schwilk and Ackerly, 2001; Pausas, 2015).

Where nonserotiny is beneficial, all that is required in the absence of fire is an efficient dehiscence mechanism of the cones/fruits that is cued once they reach maturity. Note that nonserotiny does not necessarily imply absence of fire adaptations. For example, it is coupled with thick bark (trees, Pausas, 2015) and/or resprouting (shrubs – subshrub geoxyles) among plants in low vegetation where fire is very frequent (savannas) (Lamont, He and Pausas, 2017a).

Selection will also occur at the seedling phase: young nonserotinous taxa should thrive interfire, and serotinous taxa should thrive postfire (Table 1, Fig. 3). Selected traits linked to serotiny include heat-stimulated germination, tolerance of high pH (ash), charates and soil/litter burial (as a result of postfire shifting of seeds and debris by wind and rain), high light and intra/inter-specific-competition tolerance (Table 1). Weakly or nonserotinous taxa display the reverse traits. Rarely, the germination of seeds of some species is promoted by smoke (Brown and Botha 2004) that coincides with the postfire environment, though it is usually confined to soil-stored seeds. The abundance of postfire litter microsites packed with seeds and seedlings is a two-edged sword: they both attract and satiate granivores and herbivores so that the effect on net recruitment needs to be considered. Nevertheless, recruitment in the burnt sites always exceeds that in the unburnt (Cowling and Lamont, 1987; Hanley and Lamont, 2000; Causley et al., 2016). Many serotinous seeds are black, brown, mottled or pale that implies a mimicry function against the postfire ash and/or charcoal-filled litter beds (Fig. 1H). Postfire released seeds can merge with the soil surface background to potentially deter vertebrate granivores from consuming the seeds (Saracino, D'Alessandro and Borghetti, 2004; Lev-Yadun and Ne'eman, 2013; Midgley et al., in press). To what extent these colours and patterns differ from nonserotinous seeds in the same taxon (Lev-Yadun and Ne'eman, 2013) or in different taxa, or are more effective at reducing granivory, awaits detailed study.

GENETIC ASPECTS

Genetic control of serotiny

(Figs. S1, 4)

There are two issues to consider in understanding the genetic basis for serotiny: a) that it is a heritable trait (or syndrome of traits), and b) how the selection process works at the gene level. There is much interest in the heritability (versus environmental control) of serotiny with the results so far varying from strongly to weakly heritable (Parchman et al., 2012; Budde et al., 2014; Vincenzi and Piotti, 2014; Castellanos, González-Martínez and Pausas, 2015; Feduck et al., 2015). Part of the explanation for the variable results is that serotiny is gauged in most of these studies as the percentage of cones that are closed, which clearly has an age/size/growing-condition component.

Thus, younger/faster-growing plants will have a greater fraction of young cones and register a higher level of serotiny unrelated to its genetic basis. Equally, old plants may predominantly possess open fruits/cones and thus their serotiny level is underestimated. This problem is overcome by using the slope measure of serotiny that is unaffected by annual crop size and ignores years once they are fully open (Cowling and Lamont, 1985a) – this distinguishes the 'apparent' level of serotiny (the fraction of cones/fruits that is closed) from the 'true' level of serotiny (the rate at which cones/fruits open).

Common-garden trials are an alternative approach with much to commend them as they dispense with confounding due to environmental effects (though the more common-garden locations used the better). Harris (2002) assessed the current crop of capsules in 2.5-y-old shrubs of *Leptospermum scoparium* (Myrtaceae), grown in an experimental garden at Canterbury, New Zealand, that had split to release their seeds. Populations varied in the level of splitting on a proportionate score by up to ten times, confirming that serotiny has a strong genetic basis and implying rapid adaptive responses to different fire regimes within a species. Heritability could not be determined as within-population variability was not assessed. Ideally, the assessment would be applied over a number of years in such trials to reduce seasonal variability effects.

The best study to date is that of Hernández-Serrano et al. (2014) that showed a 17 times difference in closed to total cones for 29 provenances of *Pinus halepensis.* This could be related to the level of summer drought as a surrogate for fire intensity (likelihood of fire reaching crowns of the trees). Care was taken to omit the youngest cones as these are immature while the trees were at an active stage of growth having produced cones for 10–14 y. Since cones can remain closed for 15 y the problem of fully open crops was avoided. Heritability (h^2) was moderate at 0.20. Since total or annual cone production were not used as covariates, the possibility that differences among these attributes accounted for some of the variation in serotiny remains. Using single nucleotide polymorphisms (SNP) markers for estimating relatedness among individuals in wild populations of this species, Castellanos et al. (2015) obtained a lower h^2 of 0.10, which confirms that this measure of serotiny responds to both environmental and genetic effects.

Further support for the strong genetic basis of serotiny has come from a genome-wide association studies for the variably serotinous shrub/tree, *Banksia attenuata*. Among the 382,287 genome-wide single nucleotide polymorphisms SNPs obtained, 220 were associated with the level of serotiny ($p < 0.010$, corrected for multiple tests) (Fig. S1), possibly representing 20–30 genes. It is not clear what genes are involved in controlling serotiny in *B. attenuata*, as the *Banksia* reference genome has not yet been annotated. The detection of such a large number of SNPs associated with serotiny indicates that on-plant seed storage is genetically controlled, and therefore strongly heritable in this species.

Individual trees of some *Pinus* species may be serotinous or nonserotinous (Teich, 1970, Givnish, 1981; Talluto and Benkman, 2013) indicating that this trait may be under simple Mendelian control. However, for the great majority of serotinous species, including most pines, serotiny is a question of degree, depending on thickness and density of the protective tissues, constitution of the binding resins, and the like (Fig. 1). This implies many genes control the different traits described above and many alleles for the same trait (Budde et al., 2014; Parchman et al., 2012; Hernández-Serrano et al., 2014). The effects will be additive or synergistic, including heterosis and epistasis. As alleles controlling serotiny/pyriscence (seed store and the fire cue for seed release) accumulate, so

serotiny will strengthen and seed release will increasingly be tied to fire events. A consideration of how these two options become expressed through successive generations now follows using worked examples:

1. *One gene control of serotiny*. If A is the new dominant allele for serotiny and the plants are self-compatible then 50% of seeds produced will be phenotypically serotinous. If aa (nonserotinous) are the only ones released interfire (Hernández-Serrano et al., 2013, 2014) and these perish as recruitment or maturation are now only possible if seeds germinate postfire, then 75% of seeds produced by the replacement population will be serotinous. Similarly, 88.9% of seeds produced in the next postfire generation will be serotinous (AA:2Aa × AA:2Aa). By the next generation, it will be 97.5% of seeds. Note that aa is never completely lost and can enable the population to recover nonserotiny if interfire recruitment or weak serotiny become favored. While current research indicates that this simple mechanism is unlikely since serotiny/pyriscence clearly involve multiple, coordinated traits, the same principle of progressive dilution of certain alleles via selection can be applied to multigene, quantitative allelic systems.

2. *Multiple gene control of serotiny*. To illustrate likely outcomes when serotiny is favoured, we estimated annual seed production, release and viability of retained seeds for a representative tree or shrub over 10 y since it reached maturity based on empirical trends for banksias (Lamont et al., 1994a; Lamont and Enright, 2000). The level of serotiny is controlled by the type of alleles present at a number of gene loci that we refer to as the 'gene support for serotiny', S, representing the fraction of the genome devoted to supporting serotiny that is 'saturated' (Fig. 4). Two initial gene supports for serotiny were used, 0.3 (30% of genes for serotiny are in a homozygous state) and 0.5. Two degrees of serotiny were used, 4.0 (seeds held for up to 4 y) and 12.0 (seeds held for up to 12 y), based on 100/*b* where *b* is the linear slope of fraction of seeds retained or fruits/cones closed per year (Cowling and Lamont 1985a, Lamont 1991). Progressive seed store, $H = \Sigma_{1}^{X}$(seeds produced × fraction of seeds retained × viability) where x is the number of years prior to, but including, the current year, and progressive total gene support, $G = \Sigma_{1}^{X}$(seeds produced × S × viability). It is assumed that seeds released are genetically nonserotinous (Hernández-Serrano et al. 2013, 2014) so that G gets reallocated to the retained seeds. The exceptions are when the fraction of seeds retained (R) is less than S when the genetic support is reduced to the value of R as some of S would have been lost in the released seeds. Mean S_x per seed = G/H over the nominated time period x. An example of the procedure and outcomes is given in Table S2. The process was repeated for three successive postfire generations burnt bearing a 10-y crop (strongly serotinous) to obtain new estimates of S_x.

Results show that serotinous phenotypes possess 4.5 to 6.2 times as many seeds as the current season's at the time of fire if retained for up to 10 y and 1.9 to 2.1 times as many if held for up to 4 y, depending on whether seed production is increasing, decreasing or steady (Fig. 4). If recruitment is a weighted lottery process (Lamont and Witkowski, 1995), then there would be 2.4 to 2.9 as many strongly serotinous recruits as weakly serotinous. Assuming that the seeds released interfire are genetically nonserotinous (and fail to contribute to the next generation following fire), then the gene support for serotiny gradually increases in the seeds retained so that S of the stored seeds is greater than the current season's crop (Table S2, Fig. 4). S increases per seed by 1.21 to 1.29 times for the strongly serotinous plants and 1.33 to 1.36 times for the weakly serotinous plants. The reason that the weakly serotinous have a slightly higher S than the strongly serotinous is because the

greater rate of interfire seed release means that the seeds remaining are more likely to be phenotypically serotinous, even though the rate among old seeds may be so high that even some serotinous structures release their seeds. While this mean rate of increase does not appear high, it escalates with each postfire generation so that only four generations are required for S to pass from 0.5 to 1.0 (when in theory the species is completely serotinous at least for that trait). Thus, once a serotinous mutant appears and serotiny is adaptive, then that allele is rapidly incorporated into each successive postfire generation. Here, selection essentially operates at the interfire stage, where already released seeds are 'wasted', not at the postfire stage that ensures the phenotypes present, which are increasingly serotinous, contribute to the new generation as a function of their contribution to the gene support. The reverse arguments hold for environments where serotiny is nonadaptive, such as fire-free habitats and frequently burnt savannas (Lamont, He and Yan, 2019b).

Fire-induced selection for or against serotiny

(Figs. 5, 6, 8, Table 2)

We assume from the outset that the fire return interval (FRI) exceeds the age to maturity. If the mean FRI exceeds age to reach maturity then both seed set and serotiny are possible (Fig. 5A); if it is otherwise (immaturity risk), then the species is non-viable under those conditions (it cannot reproduce) and serotiny becomes irrelevant. Where the typical FRI is shorter than individual longevity, then serotiny is a viable option; if it is the reverse (senescence risk), then interfire recruitment is the only option, so that serotiny will not evolve. Thus, serotiny is promoted when fire is at moderate intervals relative to the timing of life-history traits of a species (Fig. 5B), bearing in mind that nonsprouting shrubs usually complete their life cycle within < 50 y and often < 15 y, conifers and resprouting eucalypts may survive for >500 y, while clonal species may survive indefinitely (Lamont and Wiens, 2003). Where there are extreme FRI fluctuations about the mean in space or time, this may provide an opportunity for resprouters to establish even though the mean FRI is less than their age to maturity; these species will be nonserotinous. Within this time frame, whether serotiny or nonserotiny is favoured depends on a) whether or not the species can release its seeds in response to fire, b) the ratio of successful interfire to postfire recruits, and c) whether serotiny is required to build up the numbers of seeds sufficiently to ensure population viability in response to fire.

Three scenarios (sets of conditions and population dynamics) can be identified that promote strong or weak serotiny or the loss/absence of serotiny in the presence of fire (Table 2). These revolve around fire frequency (mean and variability of fire intervals) compared with plant lifespan, and the relative contribution of seeds released interfire vs postfire to the next generation (Fig. 5). The selection process begins with the release of the less serotinous seeds interfire. The pattern of interfire seed release can operate at three scales (Box 1): a) individuals within a population are not uniformly serotinous, with some releasing their seeds early (Givnish, 1981; Gauthier et al., 1996; Hernández-Serrano et al., 2013), b) the level of serotiny varies between whole cones or fruits within individual plants such that some open early (Goubitz et al., 2003; Lovreglio et al., 2007), and c) individual seeds are released at different rates within a given cone that collectively open at the same rate (Cowling and Lamont, 1985). Whole-plant differences in level of serotiny have an obvious genetic basis (Hernández-Serrano et al., 2014) and must constitute the dominant effect. Within-plant and within-cone differences seem much less likely to have a genetic basis: somatic mutations are only a remote possibility among old plants (Lamont and Wiens, 2003). Maternal control that might involve

recognition of the offspring genotype is possible, although this mechanism has received little direct study (Hudson, Ayre and Ooi, 2015). Epigenetic effects that might be related to position or production sequence aspects are also possible where late seed release might imply a more serotinous genotype via epigenetic inheritance (Lind and Spagopoulou 2018). Altogether, the many findings that the postfire population is more serotinous than the prefire population imply rapid genetic change between generations (Goubitz et al., 2004; Raffaele et al., 2016), even though little is known about the genetic mechanisms.

If recruitment conditions are poor through lack of vegetation gaps then these seeds are wasted (Causley et al., 2016). If the recruits survive they will be depauperate, as they are poor competitors for scarce resources, vulnerable to herbivores and often beneath adult plants, so rarely contribute seeds to the next generation (Hanley and Lamont, 2001; Fig. 9). Besides, they will be younger than the parents when fire does occur so that their seed store will be minimal or they may not even have reached maturity by then. Strongly serotinous seeds are retained on the plant and are thus more likely survive fire, and contribute more seeds to the next generation under the superior postfire recruitment and growing conditions (Goubitz et al., 2003; Causley et al., 2016). Increased serotiny in the new stand is thus due to the fact that a) seeds from less serotinous cones/fruits/individuals are released interfire so are not available to establish in the postfire microsites, and b) seeds released postfire from the more serotinous cones/fruits/individuals are more likely to lead to fecund recruits. Thus, the new cohort will be more serotinous than its parents.

When fire is rare, most seeds in serotinous cones/fruits are retained by their parent plants so that they will not be available to germinate interfire, whereas seeds released from nonserotinous structures will be available to germinate (even if seedling recruitment is low). Selection may be towards such traits as seeds that can penetrate litter, germinate quicker or have greater heat tolerances that facilitate establishment in vegetation gaps or shade (Susko, and Lovett-Doust, 2000; Hanley and Lamont, 2001; Calvo et al., 2016). These populations will trend towards weak serotiny. Where fire is expected every 1–5 years among fire-tolerant resprouters (Fig. 5), there is little difference in germination and recruitment conditions between years, and the optimum may even shift from postfire to those years of, say, above average rainfall, independent of the fire event. Here, serotiny is nonadaptive, and may even be maladaptive if it involves physiological and antigranivory costs (Lamont and Enright, 2000; Cramer and Midgley, 2009; Tonnabel et al., 2012, Talluto and Benkman, 2013). Individuals that only release seeds postfire will be selected against while selection will be towards seed release at maturity, rapid germination (no dormancy) and fast growth rates, including rapid development of below-ground, bud-storing structures (Pausas et al., 2018). Thus, with ongoing population turnover, strongly serotinous individuals are replaced by (genetically) nonserotinous individuals. By understanding the fire regime, life-history traits and relative interfire/postfire recruitment conditions, we can see how fire may select for either serotiny or nonserotiny under different circumstances (Fig. 5).

These processes are modelled in Fig. 6 in terms of the contribution of interfire recruits to the total population just before fire (from 0 to 100%). At 50%, there are as many interfire recruits as postfire and there is no selection for or against serotiny (Fig. 5C). This might be viewed as the initial state of the population. The level of serotiny is controlled by the type of alleles present at a number of gene loci (gene support for serotiny) and at this point the support is moderate. The key step is the

fraction of interfire to total individuals. Essential are the presence of crown fire at low frequency (but within the plant lifespan), superior recruitment conditions postfire, and the relevant genes and alleles for on-plant seed storage, protection and fire-cued release (though not yet in a homozygous state). The contribution of interfire recruits then falls and the gene support for serotiny escalates with each successive generation. The rapidity of this change depends on the rate of generation turnover. The converse involves the presence of frequent fires, fire tolerance or avoidance by the incumbent plants, optimal recruitment conditions not necessarily immediately postfire, and the relevant genes and alleles for seed release at maturity. The contribution of interfire recruits then increases and the gene support for serotiny decelerates with each successive generation. Under opposing selection conditions, the traits move in opposite directions as they gradually attain a homozygous condition until a new equilibrium with the selective environment is reached or the gene support is saturated in the absence of further beneficial mutations. Note that the changes in gene support did not need to involve further beneficial mutations (though this would hasten the process) – it is essentially a case of phenotypic selection.

Fig. 7 summarizes the sequence of steps and associated selective forces operating at each step over the life cycle of a given species. Note how the traits listed in Table 1 are integrated here to produce an adaptive reproductive syndrome centered on serotiny or nonserotiny. The cycle can be repeated when interfire recruitment is favored and followed by strong interfire seed production and release in the absence of fire that eventually leads to nonserotiny. Alternatively, seeds are released both postfire and interfire and depending on the ratio of postfire/interfire success rate of individuals in each phase so the trend is towards strong serotiny (high ratio) or weak serotiny (moderate ratio) or nonserotiny (low ratio). With each cycle, the genetic support for serotiny or nonserotiny and associated traits build up (Fig. 6) until an equilibrium is reached for those particular growing conditions and fire regime, where the relevant loci are in a homozygous condition and no further mutants are available for genetic change and fixation. The rate of change depends on the alleles available for fixation and the rate of generation turnover. Thus, Ne'eman, Goubitz and Nathan (2004) argued that, even if fires were rare prior to occupation of the Mediterranean Basin by modern humans 6,000 y ago, this was more than enough time for *Pinus halepensis*, at 125-y life cycles, to develop the fire adaptive traits, such as moderate serotiny, that it displays today.

The extra protection associated with serotiny indicates that there may be physiological, and thus ultimately fitness, 'costs' that need to be taken into account when assessing the level of serotiny that eventuates from selection by fire (Lamont and Enright, 2000). These involve extra water, carbohydrates and mineral nutrients but the increased demand has been shown to be minor or non-limiting (Cramer and Midgley, 2009). More generally, the costs of alternative options, such as soil storage, are unknown, the supporting structures may be multifunctional, e.g., woody fruits can photosynthesise when young and they are a source of mineral nutrients for the developing seeds (Groom and Lamont, 2010) so that their net cost is unclear, and the identity of the limiting resource that needs to be measured as a cost is unknown (Lamont et al., 2016a). One approach would be to determine experimentally the number of seeds sacrificed to achieve a certain level of serotiny, since increasing seed availability is the ultimate function of serotiny. This has only been undertaken at the simulation modelling level so far (Enright et al., 1998a, Tonnabel et al., 2012). The apparent costs of serotiny pale by comparison with the resources 'wasted' through failure of seeds released interfire to

contribute to the next generation or the gradual attrition of postfire individuals through adverse growing conditions. Thus, it may take up to 200 fire-released seeds to replace a prefire adult *Banksia hookeriana* at 15 years (Lamont, Marsula, Enright and Witkowski, 2001).

SPATIAL ASPECTS

Intraspecific variation in serotiny

(Table 3, Fig. 8)

We now consider actual examples of the outcome of the selection processes described above, first at the intraspecific scale and then at the interspecific, intrageneric, intracladal and finally, global, scales. Geographic location effects on intraspecific serotiny are highlighted in Table 3. Here, size of two *Banksia* species varies greatly between mesic sites, where most trees are tall and open and often escape ignition from ground fires, and xeric sites, where plants are shrubbier and dense and their crowns are invariably burnt by all fires. Plants at the xeric sites produce and store many more seeds and their degrees of serotiny are 50% greater. This is a genetic (ultimate) response resulting from a long history of selection, consistent with the reduced likelihood of successful interfire recruitment in the drier region and greater chance of fire-caused death of adults. In each region, when growing at the edge of roadways, where water and nutrient availabilities are higher, their size and seed stores are similarly greater than those deep in the undisturbed parts of the reserve. This is a physiological (proximate) response without selection that results in more seeds available for recruitment in both regions. However, serotiny levels (based on 100/*b*) are unaffected by these differences in growing conditions, attesting to the stability of the index and genetic basis of the level of serotiny there. Note that seeds stored as a fraction of seeds produced are greater under the better growing conditions because seed production is increasing at a faster rate such that younger (more serotinous) seeds contribute more to the total crop. Similarly, two species of pine at the mesic and xeric extremes of their climatic range have different levels of serotiny (Table 3). Here, age of the oldest closed cone is used as the index, with serotiny at the warmer coastal sites subject to more frequent crown fires twice that at the more elevated, cooler sites.

Biotic as well as abiotic interactions may affect the level of serotiny at the intraspecific level. For example, the red squirrel, *Tamiasciurus hudonsicus*, impacts on the level of serotiny among disparate stands of *Pinus contorta* subsp. *latifolia* (Fig. 8). The squirrels are most attracted to serotinous cones as they are always available; they remove the cones and store them intact preventing their seed release and dispersal in response to fire (Talluto and Benkman, 2013). Thus, selection pressure is against the expression of serotiny and this pressure increases with greater abundance of the squirrel. Relatively young trees are more likely to produce serotinous cones, so that 50% of trees can be serotinous in the absence of this granivore but < 10% when it is abundant. Old trees have little propensity to produce serotinous cones (or, alternatively, most of their cones will have released their seeds) so that their inherent level of serotiny is low and squirrels have only a minor impact on this level. Assuming that the genetic mechanism operates at the individual tree scale (it requires trees recorded as nonserotinous did not in fact result from earlier removal of all serotinous cones by squirrels), then there is support for an adaptive response by this pine towards weaker serotiny and the early release of seeds, reducing the opportunities for hoarding and consumption of seeds by this major granivore. Weak serotiny will be adaptive here as substantial

interfire recruitment is possible. Where it is not, then local extinction of heavily predated populations becomes a risk.

Interspecific variation in serotiny

(Fig. 8, Table 4)

We have demonstrated site effects (differing fire regimes and levels of granivory) on selection for serotiny within a species. The differential response of individual species to the same level of selection is even more marked. Thus, the root-suckering *Banksia elegans*, whose follicles almost never open even when removed from the parent plant (Lamont, 1988), co-occurs with the fire-killed *B. prionotes* that forms a multi-aged woodland at Lake Indoon, SW Australia (>85 times difference in degree of serotiny based on the slope index; Enright and Lamont, 1989). This involves inherent disparities between spontaneous interfire seed release (both show complete follicle opening in the presence of fire heat), and interfire recruitment patterns. Thus, weakly serotinous species show almost annual recruitment [with the peak age classes varying markedly between species and sites (Fig. 9), possibly representing differential responses to wetter and drier years, especially summer rainfall (Enright and Lamont, 1992)]. This is a function of both the greater availability of seeds annually compared with the strongly serotinous species and biological differences such as smaller seeds able to penetrate the litter and greater drought tolerances (Hanley and Lamont, 2001). Even so, interfire recruits remain subordinate to postfire recruits in this system and contribute few seeds to the postfire generation that is compensated for by the fact that annual production by the postfire plants is high. Weak serotiny is only an effective 'bet-hedging' strategy when some fires occur at intervals exceeding the lifespan of the species (Enright et al., 1998a). Alternatively, some seeds can deposit in unvegetated gaps through spatial heterogeneity of the plant cover that assists their recruitment. This remains much more likely after fire (Lamont, Witkowski and Enright, 1993) as most interfire seeds disperse around their parents and compete directly with them (personal observations).

Various habitat-vegetation types dictate the fire frequency, fire type, interfire and postfire recruitment opportunities and maximum plant lifespan that are possible (Table 2). Thus, particular species are constrained to specific habitat types. When fire frequency is ranked from nil to high, the level of serotiny increases to a maximum at low-moderate frequency and then diminishes. Different species, often within the same genus, will only be adapted to a few, often just one, of these fire-regime/environmental combinations, depending on their life-history traits (maturity age and plant longevity, Fig. 5). Either the species responds genetically to all of these agents of selection or they are excluded. Note how nil/weak serotiny can exist in contrasting habitat/fire classes, but that strong serotiny is more environmentally constrained. It is remarkable that often there is sufficient genetic diversity within a single clade for different congenerics to occupy all five fire-frequency classes recognized here, given sufficient time to adapt.

Intracladal variation in serotiny

(Table 5)

We have noted how serotiny may vary greatly within (Table 3, Fig. 8) and between (Table 4, Fig. 9) species living in different fire-related habitats. We now consider how levels of serotiny are distributed within five clades (where at least some of their members are serotinous) in relation to fire type and

whole-plant fire response (killed or survives). Is selection for serotiny always tied to fire type (no fire, surface or crown fire) or coupled with the type of fire response? If serotiny is a fire-related phenomenon, then, if the habitat is non-fireprone or only subject to ground fires (trees whose reproductive structures cannot be reached by fire), then serotiny should be absent; and this is what we find when we look at five key clades with serotinous species (Table 5). [That insufficient flame height is not the full explanation is indicated by many subshrub geoxyles in grasslands whose crowns are burnt every time there is a fire.] This is consistent with chronogram analyses that show the presence of fire is a pre- or co-requisite for the evolution of serotiny (Lamont and He, 2017; Lamont, He and Yan, 2019b). Serotiny will be confined to crown-fire habitats but not necessarily all species within a clade as a) some have adequate annual production for self-replacement upon death should a fire occur, or b) the probability of fire occurring between maturity age and longevity of the species is too small to guarantee seed release by fire in that time (Enright et al., 1998a; Keeley and Zedler, 1998; Fig. 5). Again, this is what we find (Table 5).

Since resprouters, and other fire-resilient species, do not rely on seeds for persistence in the postfire stand, one might predict that serotiny is a relaxed condition in these species: this is not what we find. In the long-term, fitness and genetic diversity of resprouters is just as dependent on seeds as nonsprouters and their levels of serotiny may be similar (Enright et al., 1998b). This might be exacerbated by their typically low annual seed set compared with nonsprouters (Lamont and Wiens, 2004; Pausas et al., 2004. Thus, selection tends to favor serotiny among resprouters almost as much as among nonsprouters (Yate's χ^2 test for global figures: $P = 0.0344$ for hypothesis that nonsprouters > resprouters, $P = 0.0688$ for hypothesis that there is no difference).

TEMPORAL ASPECTS

Evolution of serotiny and nonserotiny among genera

(Figs. 10, 11)

We now consider the evolutionary history of serotiny and nonserotiny among three well-studied clades: *Hakea* (Australia), *Pinus* (Northern Hemisphere) and *Protea* (Africa). The onset of serotiny in *Hakea* is associated with departure of the ancestral parent from rainforest into fire-prone sclerophyll woodland, 18–20 million years ago (Ma) (Fig. 10). Diversification of moderately serotinous species escalated in the mid-late Miocene, possibly associated with the advent of a mediterranean climate in SW Australia (Lamont and He 2017) with its intense, moderately frequent, summer-autumn fires. The appearance of much woodier and more strongly serotinous fruits 5 million years (My) after origin of the clade can be attributed to selective pressure from the increasingly abundant granivorous black cockatoos (*Calyptorhynchus*) (White et al., 2011) in the face of the increasing advantages of serotiny for seedling recruitment as the climate became drier and more seasonal (Lamont et al., 2016a). Weak serotiny originated at much the same time but diversified more slowly at first then escalated from 7 Ma with smaller fruits that tended to stay green, mimicking the leaves and branchlets and remained embedded among the spiny leaves. This, in turn, was an adaptive consequence of the large fruits arising from exposed, bird-pollinated flowers, such that mimicry and crypsis, available to insect-pollinated flowers concealed within foliage, were not options (Hanley, Lamont and Armbruster, 2009). Some lineages were successful at interfire recruitment (Hanley and Lamont, 2001) while others by now had spread into sparse vegetation (arid or saline) or summer-rainfall savannas (Lamont et al., 2016b) where flames were unlikely to reach the crown, both promoting weak/nonserotiny. The only

species to complete the evolutionary cycle by occupying non-fireprone rock outcrops and becoming nonserotinous is *Hakea clavata* that arose about 6.5 Ma.

Serotiny arose in *Pinus* ~113 Ma from nonserotinous congeneric ancestors subject to surface fires over the previous 25 My, and coincided with the advent of crown fires that could now reach the cones and stimulate seed release (Fig. 11). Diversification of the serotinous lineage (subgenus *Pinus*) did not occur until 50–45-Ma or else speciation was balanced by extinction up until then. As diversification of non-fireprone, nonserotinous lineages escalated from the mid-Paleogene and into the Neogene-Quaternary, peaking over the last 5 My, so too did the fireprone, serotinous lineages but at a lower rate. Six species lost serotiny on entering fire-free habitats over the last 25 My. While a nonserotinous lineage (subgenus *Strobus*) developed wingless seeds 50–45-Ma (Fig. 11), diversification only began 30–25 Ma, increasing linearly at a low rate until the present associated with dispersal by granivorous birds (Corvids) and squirrels. Since winged seeds are part of the serotinous syndrome (He et al., 2016a), a return to serotiny remains possible for most of the currently non-fireprone pines should the fire regime change, but this becomes less likely for these species as wingless nutlets are now entrenched in their genome.

Protea was serotinous from the time of its separation from nonserotinous relatives 28 Ma in the SW Cape of South Africa, and diversification escalated there from 20–15 Ma (Fig. 11) possibly coinciding with the advent of a predominantly winter-rainfall climate and summer-autumn fires at moderate intervals (Lamont and He, 2017). As the clade migrated north and east to the summer-rainfall zone in the 15–10-My period, one lineage adopted both resprouting in response to fire and loss of serotiny and could now enter this zone where it diversified strongly. Resprouting is required to survive the frequent (often annual) winter-spring fires and serotiny is redundant or even maladaptive if it involves a fitness cost. Thus, serotiny has gone in opposite directions in the same genus as determined by the contrasting fire regimes in different regions. A general picture emerges among these three clades of a) nonserotinous ancestors in non-fireprone habitats (or surface fires in the case of pines), b) initiation and proliferation of serotiny with the introduction of crown fires, and c) loss of serotiny with migration to (almost) fire-free habitats or greatly expanded summer-rainfall grasslands/savannas with frequent fire, but diversifying at a much lower rate than the serotinous lineages.

Evolution of serotiny and nonserotiny among families

(Figs. 12, 13)

Moving from the genus to family scale shows how serotinous and nonserotinous genera can arise independently numerous times within the same parent clade (Fig. 12). Each of the ten serotinous lineages in Proteaceae commences in a fireprone environment but not all fireprone environments lead to serotiny. Soil storage (geospory) is diagnostic for 17 lineages that requires heat or smoke to break dormancy. Why one fire-response type is selected for rather than the other is unclear, but it must involve possible differences in the selective fire regime and postfire recruitment conditions as well as phylogenetic constraints. One lineage (*Faurea*) lacks any storage and occurs in savanna (highly fireprone) or rainforest (non-fireprone). In addition, the serotinous structures are matched (same ontogeny) at the fruit level within each subfamily (homologous) but the fruit types are

quite different between subfamilies (analogous – homoplasious). Thus, the Proteoideae has indehiscent achenes while the Grevilleoideae has dehiscent follicles.

Further, the infructescence types are non-homologous within each subfamily. Thus, *Protea* has a capitulum plus an involucre of bracts that wrap around the entire head of fruits, while in *Aulax* and *Petrophile* each fruit has its own scale attached to a rachis (multiple rachises in the case of *Aulax*). Among the five fireprone, Grevilleoid lineages, *Banksia-Dryandra* alone has a compound fruit with persistent bracteoles. In addition, its follicles rarely open on severance of the cone from its vascular supply but the serotinous structures of all other lineages release their seeds when their vascular supply ceases. Further, the various serotinous structures originate at quite different times (Lamont and He, 2012). Thus, the serotinous cones of *Aulax*/*Petrophile* arose at least 75 Ma while those of *Leucadendron* appeared 50 Ma, assuming that the cones were serotinous then based on ancestral trait assignment techniques (Lamont, He and Yan 2019a). *Banksia* was serotinous from its beginnings > 60 Ma while the serotinous follicles of *Hakea* can be dated to 20 Ma (although the node is set where it separated from *Grevillea*, and the complex is at least 35 Ma so it may be older). The issue of the origin of particular serotinous structures therefore cannot be separated from the reasons for the origin of particular lineages in geological time that is steeped in their fire history (Lamont, He and Yan, 2019a).

At an even broader global scale, serotiny has arisen independently among 12 families throughout the phylogeny for seed-bearing plants (Fig. 13). This covers a time span of almost 150 million years, coincident with the history of fireprone floras over that period, with serotiny arising well before, or coincidentally with, the onset of fireproneness (Lamont and He, 2017). Beginning with two conifer families (Taxodiaceae is embedded in Cupressaceae in this phylogeny), then proceeding to the monocots (two families – Lyginiaceae is embedded in Restionaceae in this phylogeny). This is followed by two 'old' Eudicot families, Proteaceae and Myrtaceae, then a moderately old family, Casuarinaceae, and finally the modern Eudicot families, Ericaceae, Bruniaceae and Asteraceae. Each of the ten families begins with nonserotiny and serotiny arrives later, sometimes early (Anarthriaceae, Bruniaceae) and sometimes only recently (Ericaceae). Note that hundreds of other clades are fireprone apart from the serotinous ones but they have quite different adaptations for fire survival.

Overall evolutionary history of serotiny

(Fig. 14, Table S3)

Collating the 31 records available for the ancestral condition of serotiny shows that it has arisen in every 10-My interval over the last 110 My (Fig. 14). Three peaks may be identified: a small peak towards the close of the Cretaceous corresponding to the origin of many extant families and their early radiation, and two larger peaks in the Cenozoic: the Paleocene–mid-Eocene and Oligocene–Early Miocene. The drop in trait diversification and proliferation rates in the 80–70 and 40–30-Ma periods corresponds to local depressions in charcoal deposition, temperature and atmospheric O_2 (He and Lamont, 2017). Of particular note is the sharp drop of 4°C at 34 Ma corresponding to the completion of the Antarctic circumpolar current at that time (as Australia finally broke away) that correlates with the dip in O_2 levels and no doubt decreasing incidence of fire as a selective force. The peaks at 60–40 and 30–0 Ma cannot easily be attributed to any increase in charcoal deposits but they do correspond to the Early Eocene Thermal Optimum

(55–45 Ma) and Mid-Miocene Thermal Optimum (17–14 Ma) with their expected positive effect on fire activity and the evolution of fire-adapted traits. Clearly, seasonality began to take control of the fire patterns as O_2 levels started to fall from 30 Ma. Conversely, since evolution of serotiny/pyriscence requires fireprone conditions, these patterns of speciation can be used to imply the prevailing levels of fire activity as experienced by these clades through time, independent of charcoal data that will always be an inadequate and biased record of the occurrence of fire beyond wetlands where fossils are best preserved (He et al., 2012).

It is noteworthy that a recent independent analysis of the subfamily Callitroideae, Cupressaceae, produced much the same pattern with geological time (Crisp et al., 2019; Fig. 14). The exception was the marked diversification of nonserotinous taxa over the last 10 My that can be attributed to both diversification of non-fireprone species and entry into savanna-type habitats. Novel fire-adapted traits may appear in response to the imposition of an initial fire regime (the outcome of primary directional selection) and be replaced later by a change in the fire regime (secondary directional selection) or be retained in the daughter lineage while non-fire-related traits arise in response to other agents of selection (stabilisation) (Lamont, He and Yan, 2019a). Overall, the initiation/(loss) of serotiny coincided with the simultaneous origin of the genera or clade and advent of fire-proneness in 31% of cases, millions of years later due to a change in the fire regime in 29% of cases, and serotiny was retained (stabilized) as continuing to be adaptive in 32% of cases. The evolution of serotiny is associated with the historical occurrence of fire from non-fireprone ancestors, and its occasional absence in serotinous clades has been the result of more recent changes in the fire regime. The loss of serotiny completes the evolutionary cycle but under quite different environments than originally – there are few records so far of lineages returning to the everwet forests of their ancestors. Leptospermoid Myrtaceae may provide one exception, where trees with vestigial (blind) epicormic buds may be present in non-fireprone rainforest (Clarke et al., 2012) and these are also nonserotinous.

Over geological time, the only way to gauge the incidence and net rate of trait change is via speciation. The four well-studied clades already considered here (Figs. 9, 10, 13) are now collated (Table S3) to show enormous variation in the rates over the Cenozoic with a tendency for both serotiny and nonserotiny to peak over the last 5 million y (My) in three clades, but over the last 15–5 My in *Hakea*. The three clades whose ancestral state is serotiny have tended to favor serotiny over evolutionary time, and vice versa, consistent with their preferred habitats.

Callitroideae is fascinating for the fluctuating dominance of serotiny and nonserotiny over evolutionary time: the immediate ancestors of Cupressaceae were probably nonserotinous immediately followed by serotiny in Callitroideae then nonserotiny with a general decline in the speciation rate coinciding with the world drops in temperature and O_2 (and a likely decrease in the incidence of fire) peaking at 34 Ma (Fig. 14). This was followed by a marked increase in serotinous species in the Oligocene-Miocene, followed by greater nonserotinous speciation over the last 10 My. The greatest net rate for the evolution of serotiny over the last 5 My is 14 species/My among temperate shrubland proteas followed by 5 species/My among pines. At the same time, pines yielded 18 nonserotinous species/My and savanna grassland proteas 3.5 species/My but the reasons differ: pines migrated strongly into fire-free habitats, e,g., alpine (as did Callitroids), or frequently (understory) burnt savanna woodlands/forests, while proteas migrated into frequently burnt, savanna

shrubby grasslands only (Table 4). *Hakea* is of particular interest for speciation of the serotinous lineage far outstrips that of the nonserotinous in the Upper Miocene (contributing to this as the only time in which serotiny exceeds nonserotiny overall for the four clades) with a few species moving into non-fireprone habitats and summer-rainfall savannas in the late Miocene.

CONSERVATION ISSUES

Understanding how anthropogenic climate change influences plant reproductive ecology is fundamental to understanding how climate variation will affect plant communities in the future (Parmesan & Hanley 2015). As we have shown, the expression of serotiny depends on the interplay of fire-regime and a number of post-fire environmental conditions that dictate germination and seedling recruitment success. Despite an expectation that fire frequency and intensity will increase in Mediterranean-climate regions (at least until decreasing rainfall has a significant effect on reducing fuel loads), the magnitude of shifts in fire regime remain uncertain (Pausas, 2004; IPCC, 2014). The level of serotiny was higher among two Mediterranean pine species (*P. pinaster* and *P. halepensis*) in populations from areas affected by more frequent fires over the last 30 y (Hernández-Serrano et al., 2013; see also Vincenzi, & Piotti, 2014). Added to this, declining and more unpredictable interfire precipitation regimes in Mediterranean-climate regions, especially during the key winter seedling recruitment period (IPCC, 2014), will add further stress on fire-following plants with the serotinous habit in particular at serious risk of local extinction (Lamont et al., 2007; Enright et al., 2014) and possible selection for earlier reproductive maturity and increased serotiny (Vincenzi and Piotti, 2014). On the other hand, invasion by serotinous pines, with their greater seed stores, is now occurring in regions where endemic levels of serotiny are negligible (Rafaele et al., 2016).

Habitat fragmentation may also have an effect on fire regimes, with any impact on fire frequencies depending on proximity to human activity (e.g. arson) and local land-use type (Keeley et al., 1999; Syphard et al., 2007; Regan et al., 2010). Moreover, despite a considerable amount of work on the separate effects of altered fire frequencies and fragmentation on plant populations, few if any studies have considered the two factors in combination (Regan et al., 2010). Habitat fragmentation also affects serotinous species in other ways. Many of the serotinous Proteaceae characteristic of southwestern Australia and the Cape of South Africa, for instance, are bird pollinated and effective cross-pollination (in the case of *Banksia hookeriana*) often involves the movement of bird pollinators over considerable distances (Krauss et al., 2009). Although by no means unique to serotinous species, disruption of plant mating systems in fragmented landscapes represents a further problem for the many bird-pollinated species that also tend to be strongly serotinous (Lamont, Klinkamer and Witkowski, 1993). When faced with shifts in land-use, weakly serotinous or nonserotinous species may take advantage of increased disturbance. Hanley & Lamont (2001) not only showed how weakly serotinous Western Australian Proteaceae exhibited much greater interfire recruitment than their strongly serotinous conspecifics, but at least two weakly serotinous species (*Dryandra sessilis* and *Hakea trifurcata*) are common invasives in disturbed areas near roadways and tracks through reserves (M. E. Hanley, personal observations*)*. Thus, it seems likely that increased anthropogenic creation of bare areas will favour non- or weakly serotinous species over strongly serotinous species.

Serotinous species in South Africa and Australia are highly favored in the wildflower trade (Rebelo and Holmes, 1988; Lamont et al., 2007; van Deventer et al., 2016). Harvesting is usually

744 undertaken from wild populations but plantation sources are increasing (Blokker, Bek and Binns,
745 2015). Thus, 34.0% of produce exported among 170 species in Western Australia are from serotinous
746 species (Anonymous, 2018), even though they only contribute 13.8% of the total flora. Of the 41
747 species harvested in the wildflower trade in the Agulhas Plain, 76% are serotinous (van Deventer,
748 Bek and Ashwell, 2016), even though serotinous species only account for 2% of the Cape flora. The
749 conservation status of many of these species is considered threatened or vulnerable. Bloom
750 harvesting greatly reduces the subsequent size of the canopy-stored seed bank, especially when levels
751 of harvesting rise above 30% (Lamont et al., 2001; Treurnicht et al., 2017). This is especially so for
752 the fire-killed nonsprouters, which account for 80% of the harvested species (Deventer, Bek and
753 Ashwell, 2016), as this group is entirely dependent of seedlings for postfire recovery. This
754 exacerbates the likelihood of local extinction among serotinous nonsprouters that are already the
755 most vulnerable to decreasing rainfall and increasing fire frequency/intensity associated with climate
756 change (Enright et al., 2014). High levels of harvesting can also substantially increase plant
757 mortality, e.g., mortality in commercially exploited *Brunia albiflora* populations ranged 8–33%
758 compared with only 1–3% natural mortality (Rebelo and Holmes, 1988). Many serotinous species are
759 also highly susceptible to the root-rot fungus, *Phytophthora cinnamomi* (Lamont et al., 2007).
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761 RESEARCH CHALLENGES
762
763 Much has been learned about the processes controlling the biology of serotiny since the publication
764 of a major review on its ecological significance 30 years ago (Lamont et al., 1991). Lamont and
765 Enright (2000) listed worthwhile research topics that remained, some of which have been achieved
766 and others that have not. Enright et al. (2007) stands alone as an overt comparison of the relative
767 taxonomic and spatial distribution of geospory and serotiny in an entire fireprone system, concluding
768 that 95% of species exhibited some sort of storage. That serotiny might enhance genetic diversity
769 equivalent to that of the multiple generations represented by soil storage has been examined only
770 once and shown to occur surprisingly fast in a postfire population of *Banksia hookeriana* (Barrett et
771 al., 2005). It was noted then that demographic models would be greatly improved if there was a
772 sounder knowledge of plant longevity – this has received little overt monitoring since but it continues
773 to be important and there have been attempts at estimating longevity among long-lived species
774 (Merwin et al., 2012, Martin-Sanz et al., 2016). The early indications that the germination of some
775 serotinous seeds could benefit from heat pulses or survive extraordinarily high temperatures, both
776 akin to the responses of geosporous species, has been confirmed (Hanley and Lamont 2001, Tangney
777 et al. 2019). This suggests that selection for insulation from fire heat may not be the prime or only
778 function of protective structures.
779 Once it is recognized that seed serotiny is only part of a vital reproductive syndrome for
780 hundreds of species, it opens the way to examine how selective forces shape a wide array of traits,
781 not just those that control the rate of cone/fruit opening. We list 23 that appear relevant here (Table
782 1): the operating mechanism of only one is beginning to be understood in any detail (Huss et al.,
783 2018, 2019) and the genetic basis of any one of them is only understood at a crude correlative level.
784 Having identified what traits are involved in the serotiny/nonserotiny syndromes this opens the way
785 for intensive study of their genetic basis and what genes control their expression. With increasing
786 interest in the heritability of serotiny (Parchman et al., 2012, Budde et al., 2014, Vincenzi and Piotti,

2014, Castellanos, González-Martínez and Pausas, 2015, Feduck et al., 2015), it is essential that the index reflects the extent to which viable seeds remain stored on the plant over time and is not confounded by plant age, fecundity or growing conditions. The most stable index in this regard is the slope measure of serotiny but our literature survey shows that it has only been adopted as the standard measure in Australia. Indices of serotiny are most meaningful when calculated on a viable seed basis but this is almost never done. A critical look at what exactly the indices of serotiny currently available measure, and how they can be improved, is required.

A particular challenge is identifying the morphological, environmental or genetic basis for why some seeds are released early and others late (in the absence of fire) within the same population, plant or cone (Salvatore et al. 2010). Huss et al. (2017) have done an excellent job in elucidating the anatomical (but not yet chemical) basis of the differences in level of serotiny along a climatic gradient. Fruits or cones exposed to direct sunlight appear to open early (soliscence, Lamont 1991) that has yet to be formally documented, but this only occurs under special circumstances. We have assumed that the seeds released in the absence of fire are genetically nonserotinous in our models (Figs. 4, 6). What is the actual gene store for serotiny (S) among individuals in a population at increasing times since fire? One puzzle is, if they are (epi)genetically distinct, how weakly/non-serotinous and serotinous seeds are recognized by the mother plant such that the former are released early and the latter later or never? Are there within-plant/cone spatial, temporal, morphological or physiological aspects that control early or late release of seeds? This may well involve epigenetic inheritance (Lind and Spagopoulou 2018); maternal control has received insightful recent study in some species (Li et al. 2017) but has yet to be tackled in the context of serotiny (Hudson et al. 2015).

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Box 1

THE CONCEPT OF SEROTINY

The origin of the term serotiny is quite general in meaning: delayed occurrence of an event (Lamont, 1991). Thus, strictly one should refer to serotinous fruits or cones (delayed opening in release of seeds) or serotinous seeds (prolonged retention on the plant). However, this rider is hardly ever used, and reference to serotinous individuals and species is taken to imply the prolonged retention of seeds on the mother plant. "Prolonged" is usually interpreted as retention of some seeds in the presence of at least the next crop of mature seeds (Lamont and Enright, 2000). Cones/fruits, individuals, populations or species can be described as i) nonserotinous (seeds released at maturity), ii) weakly serotinous (seeds retained for 1–4 y), moderately serotinous (5–9 y), or iv) strongly serotinous (10+ y). Individual plants within a population may be assigned to any of these four categories – this may have a temporal or growing condition component (e.g., most cones/fruits might be open in moribund plants, all cones/fruits might be closed with only 1–2 y crops on them, vigorously growing plants will mostly comprise young, closed cones/fruits). Among pines and proteas, cones are usually either closed (immature, green; older, brown – some may open) or open (old, grey). This also applies to species with solitary woody fruits, such as hakeas, with similar colour changes apparent. For other clades, with clusters of fruits (eucalypts) or cones (banksias), the fraction of open fruits per cluster/cone may vary in any year's crop (Lamont et al. 1994a).

In all cases, the supporting structures gradually open and release their seeds over time, but at different rates. As the structures age, so the threshold cue required to stimulate opening becomes less intense, e.g., lower temperatures are needed (Enright and Lamont 1989), and they begin to open spontaneously. It is the rate at which this occurs, independent of crop size, that determines the 'true' level of serotiny (Cowling and Lamont 1985a). The fraction of all cones/fruits closed on a plant at a given time is an estimate of 'apparent' serotiny as any differences in annual crop size (with their different levels of opening) are not taken into account. Nevertheless, even the 'slope' measure of serotiny will be susceptible to say, increases in mean temperatures due to climate change, so that it is not possible to identify 'absolute' (genetically based) levels of serotiny – it will always have an environmental component (Hernández-Serrano et al. 2014).

In other contexts, the concept of serotiny has been widened to include seeds retained i) on a dead ephemeral plant where seed dispersal relies on dislodgement during highly stochastic rainfall events in desert environments (Martínez-Berdeja, Ezcurra and Torres, 2015) or physical dislodgement during movement as a wind-blown 'tumbleweed' (Borger et al., 2007; Long et al. 2015), ii) within the cladode of certain cacti (*Mammillaria*) that ensures the seeds become 'primed' before dislodgement by rain (Santini and Martorell, 2013), and iii) on a living plant for up to nine months beyond the point of reaching maturity with retention of heat-tolerant proteins during that time without any obvious environmental cue for release has been recorded in a few hard-seeded legumes (*Peltophorum*) (Silva et al. 2017). All these examples lack the three attributes that characterize the many hundreds of species that are the subject of this monograph: a) on-plant storage of most mature seeds for at least a year, b) retention of seeds within woody supporting structures, and c) seed release in response to fire that may have a direct or indirect role in seed release (e.g., melting of binding resins, death of the supporting branch with associated desiccation processes).

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Fig. 1. Selection of serotinous structures and their association with postfire seed release and seedling establishment. (from left to right, top to bottom). A) Pyramidal cones (strobili) of *Callitris* (*Actinostrobus*) *pyramidalis* (Cupressaceae) (15 mm long); B) Postfire globular cones of *Cupressus sempervirens* (Cupressaceae) (30 mm diameter) in Spain with scale complexes beginning to separate in order to release seeds; C) Ovoid cone of *Pinus patula* (13 cm long) in Mexico, note fibrous bark and retention of foliage that would enhance flammability around the cone; D) Postfire ovoid cones of *P. radiata* (10 cm long) from California, with reflexed scales that have allowed the release of their seeds, note how the annual clustering of cones enables their age to be determined; E) Two follicles, 40 mm long, of *Hakea cyclocarpa* (Proteaceae), whose recurving prevents the ovipositor of insect granivores from penetrating the thinnest part of the fruit; F) *Hakea stenocarpa* follicle (35 mm long) mimicking branchlet (warts and all!); G) three follicles of *H. platysperma* (65 mm long), the largest fruits of all 170 *Hakea* species, whose size and globular shape resist penetration by the main granivore of hakeas, the black cockatoo (reconstructed head on left); H) *Xylomelum angustifolium* (Proteaceae) follicle, the heaviest among all species with woody fruits, with one of its two seeds showing mottling that might serve a cryptic function postfire; I) *Lambertia echinata* (Proteaceae) follicle (15 mm wide) with sharp spines not unlike surrounding foliage*;* J) Globular cone of *Banksia lemanniana* (Proteaceae) with 12 follicles (35 mm wide) showing among persistent dead florets and leaves that enhance flammability; K) *B. hookeriana* (2 m tall) alight, showing scores of floret-covered cones alight but which survive the heat; L) Postfire *B. leptophylla* cone (85 mm diameter) showing split separator pulling two winged seeds out of a ruptured follicle; M) Cluster of corky, cup-shaped capsules (20 mm wide) of *Leptospermum spinescens* (Myrtaceae), Inset: two seeds of *Callistemon* (*Melaleuca*) *teretifolius* (1 mm long) [South Australian Seed Conservation Centre]; N) Elongated, spiral-arranged cluster of sessile capsules of *Callistemon citrinus* (Myrtaceae) (80 mm long) in NSW, Victoria [Fir0002/Flagstaffotos, GFDL v1.2]; O) Five woody capsules of *Eucalyptus todtiana* (Myrtaceae) that have released their contents on drying out postfire, three fertile seeds on the left and six aborted seeds on the right, Inset: capsules of *Angophora hispida* (Myrtaceae), 8 mm wide, already released their seeds even though subtended by current season's leaves (nonserotinous), in NSW [http://www.friendsoflanecovenationalpark.org.au/Angophora_hispida.htm]; P) 5 spikes of *Connomois parviflora* (Restionaceae) (12 mm long) each bearing one nutlet in W Cape, [Tony Rebelo, https://www.inaturalist.org/photos/15419835]; Q) *Protea burchellii* (Proteaceae) in W Cape with loose cone of dry bracts and florets (100 mm long) that burn off when ignited [Elana Mostert, iSpot]; R) fruits of *Protea burchellii*, with tuft of brown hairs (15 mm diameter), released onto an ashbed after fire; S) Cone of *Allocasuarina torulosus* (Casuarinaceae) (30 mm long) with scores of samaras emerging from pairs of woody valves when left to dry out on a bench [Ian T. Riley, with permission]; T) Globular-ovoid cone of *Isopogon trilobus* (Proteaceae) (35 mm long) with 150 tightly bound scales (bracteoles) with only a few bearing flattened, fertile nuts; U) Loosely clustered, ovoid cones of *Petrophile brevifolia* (20 mm long), sparsely scaled and bearing few nuts V) Seedlings (5–12 cm tall) from serotinous *H. polyanthema*, *B. attenuata* and *B. hookeriana* in litter microsite (see Lamont et al. 1993). Plants native to SW Australia unless otherwise indicated. E–I, L, N, O photographed by B. Lamont; B–D by J. Pausas; A, J, M, T, U by T. He.

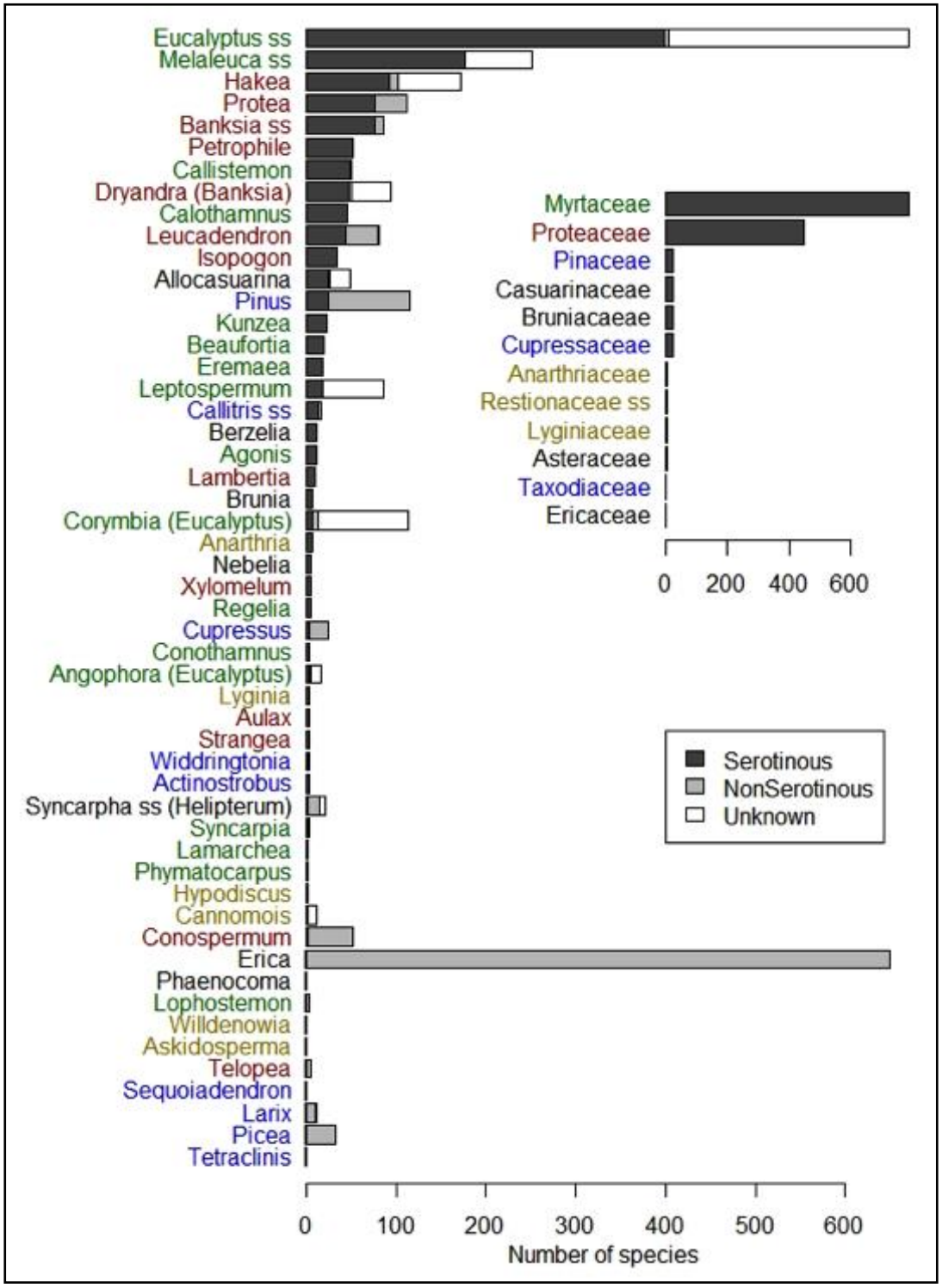


Fig. 2. Taxonomic distribution of serotiny (dark grey), and nonserotiny (light grey) in clades that include serotinous species, plus numbers of species yet to be examined in the clade (white). The large graph is at genus level and the inset is at family level. Conifers are given in blue, monocots in gold, and the rest are dicots: Myrtaceae is given in green, Proteaceae in red, and the smaller groups in black. See Table S1 for details on their geography, morphology, habitats and supporting references.

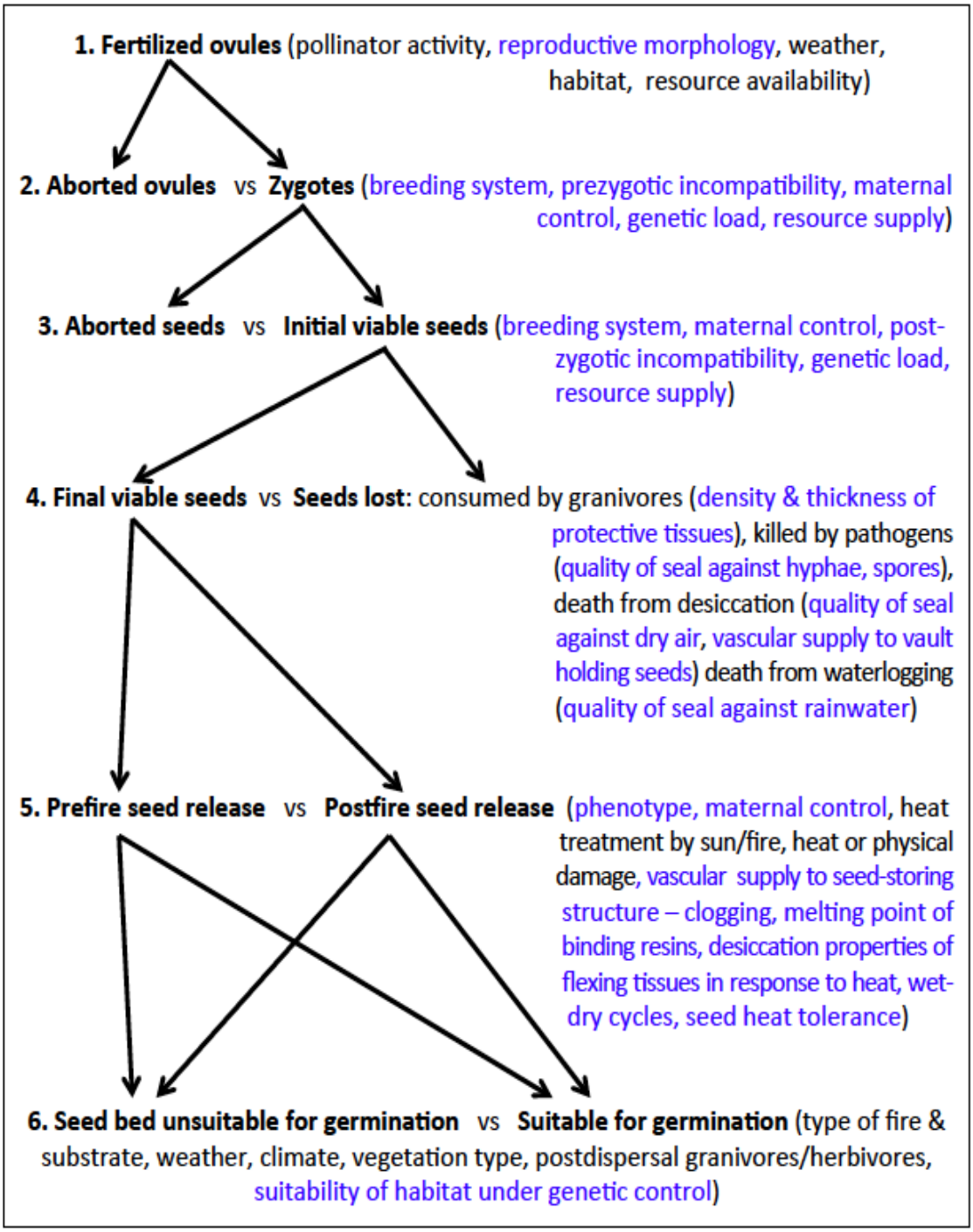


Fig. 3. Sequence of events involved in the success or otherwise of on-plant seed storage and release with the options at each step highlighted in bold. The constraints at each step are given in parentheses and those subject to genetic control are highlighted in blue. Support obtained from Ledig and Little (1979), Lamont et al. (1994a, b), Groom and Lamont (1997), Lamont and Enright (2000), Lamont and Wiens (2002), Cramer and Midgley (2009), Parchman et al. (2012), Hernández-Serrano et al. (2013), Talluto and Benkman (2013), Budde et al. (2014), Causley et al. (2016), Huss et al. (2018, 2019), Tangney et al. (2019).

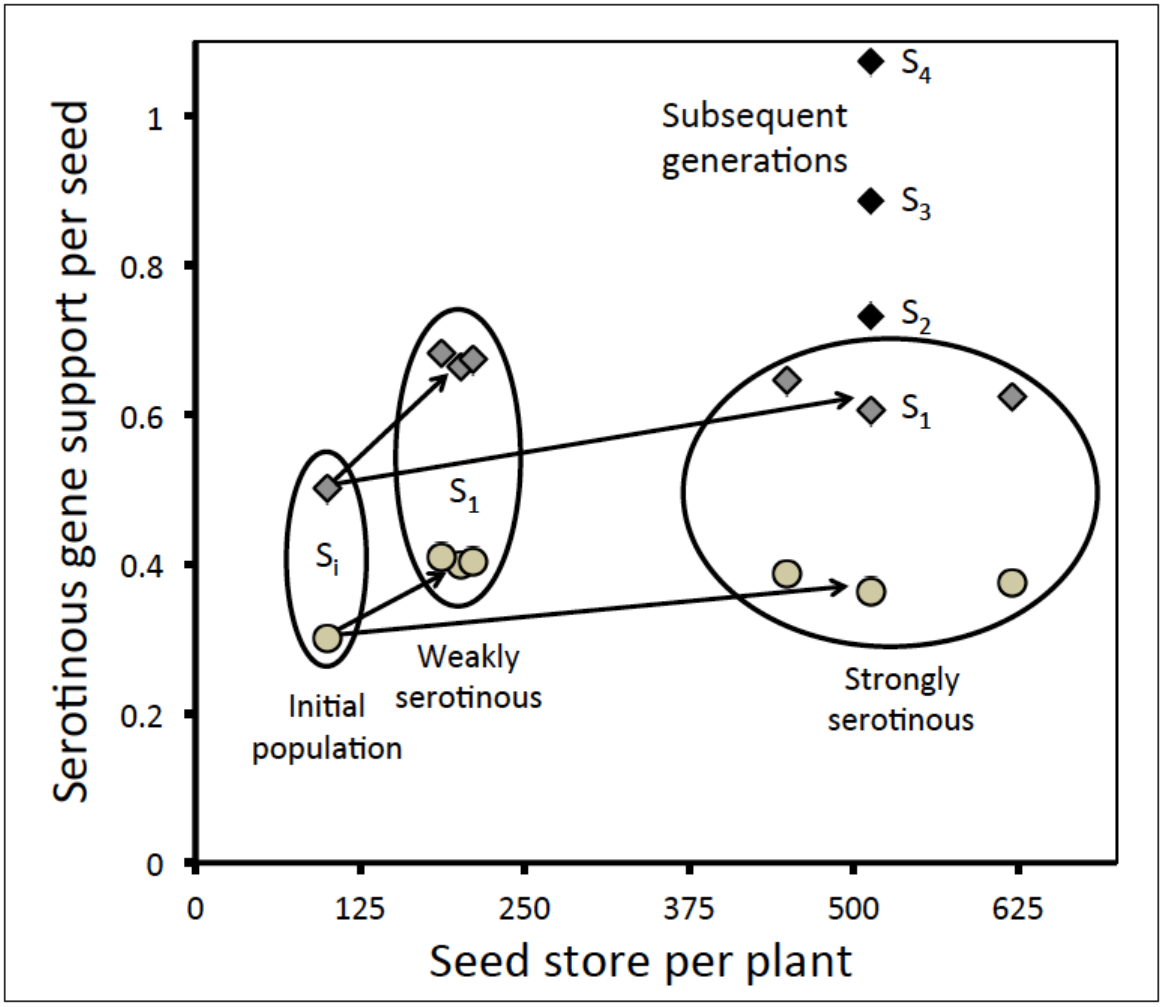


Fig. 4. Model outcomes for the impact of initial level of serotiny and increasing gene support for serotiny per seed (S) at two initial levels [S_i = 0.3 (circles), 0.5 (diamonds)] on seed storage and gene support for the next postfire generation The hypothetical plant produces 100 seeds in the initial season and may store seeds for up to 4 y (weak serotiny) or 12 y (strong serotiny) with seeds released at a steady rate from zero at y 1 (the slope measure of serotiny, Cowling and Lamont 1985). Three scenarios are modelled: decreasing (left hand values), increasing (right hand) or constant (middle) annual seed production over the ensuing 10 y. The arrows show how both sets of S_1 values (circled) increase when genetically nonserotinous seeds are released by individuals preferentially interfire. Note that S increases in each postfire population that replaces the prefire population (S_1 to S_4) – illustrated here for the strongly serotinous population with increasing seed production up to the current year, but the same trend applies to all scenarios.

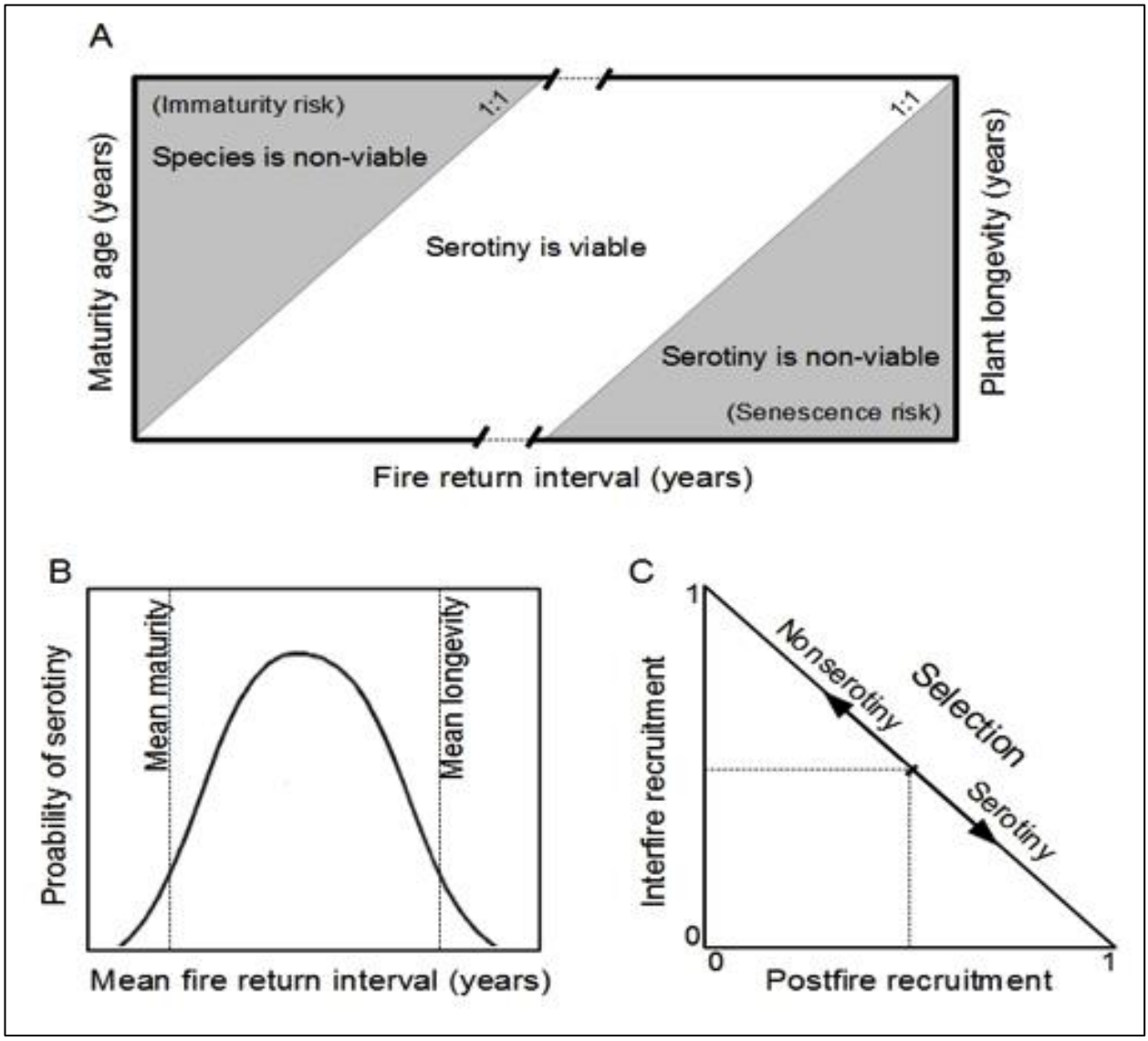


Fig. 5. Models of the effects of varying fire return intervals (FRI) and ratio of postfire to interfire recruitment success on serotiny as a viable option. A) Species and serotiny viability for FRI relative to time to reach plant maturity and plant longevity (model based on Pausas and Keeley, 2014). B) Selection for serotiny is suppressed as mean FRI approaches or is less than mean age to maturity and approaches or is greater than mean plant longevity. C) The ratio of successful postfire to interfire seedling recruitment determines whether serotiny or nonserotiny are selected for (expanded in Fig. 6).

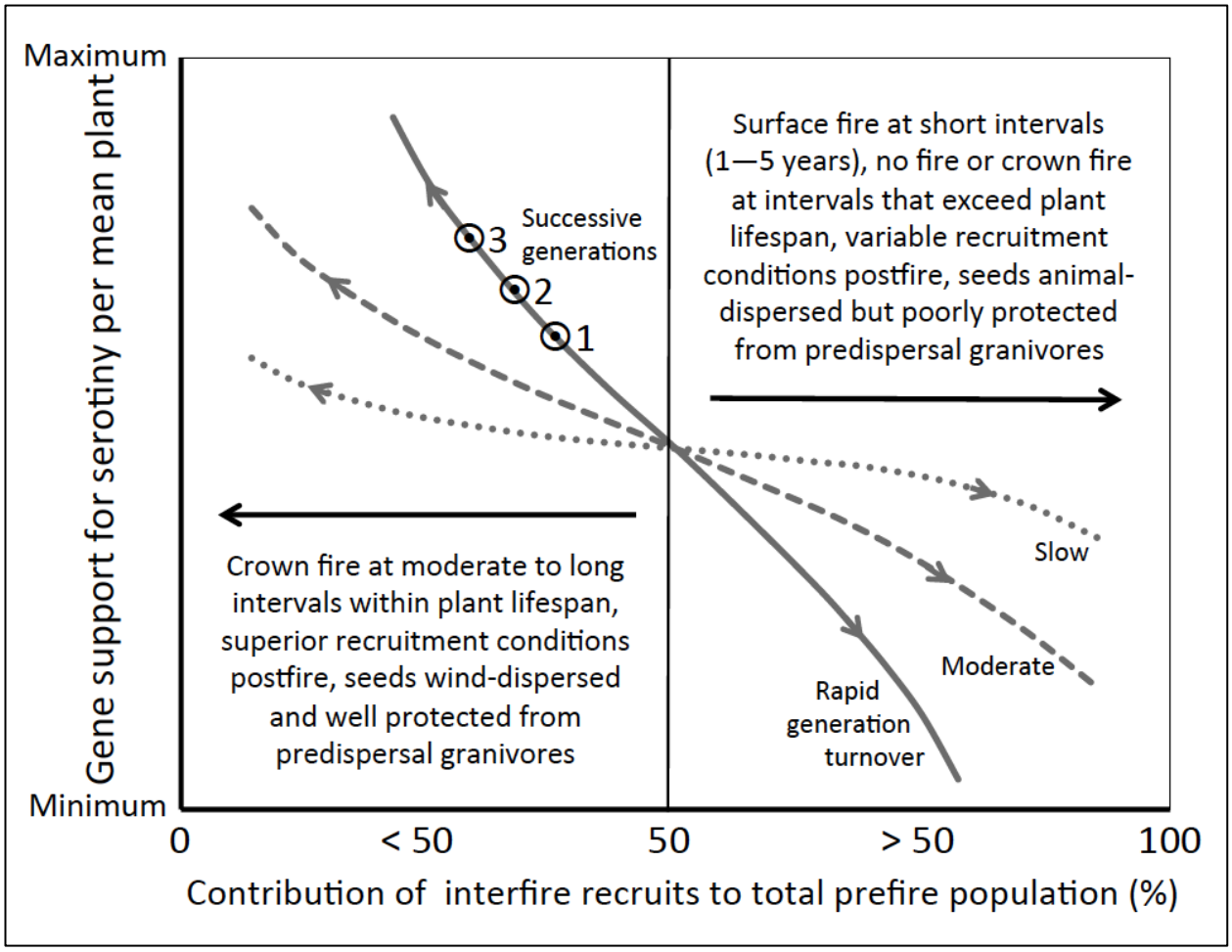


Fig. 6. Idealized relationship between gene support for serotiny per mean plant (S) and contribution of interfire (inter) recruits to the total, interfire + postfire (post), population at three rates of population turnover (R), slow, moderate or rapid. $S = R \times (S_i \pm S_i \times \text{inter/post})$. Prefire conditions are either less (left of the 50% line) or more (right of the line) favorable for recruitment over postfire. Thus, postfire-released seeds carry greater gene support for serotiny than those released prefire. Equally, prefire released seeds carry greater gene support for nonserotiny. With each successive generation, the level of serotiny will accelerate ($+ S_i \times$ post/inter ratio) or decelerate ($- S_i \times$ inter/post ratio) depending on the rate of generation turnover until an optimum is reached. Modelled on data in Givnish (1981), Cowling and Lamont (1985a), Lamont et al. (1991), Gauthier, Bergeron and Simon (1996), Enright et al. (1998a, 1998b), Lamont and Enright (2000), Goubitz, Ne'eman and Nathan (2003), Parchman et al. (2012), Talluto and Benkman (2013), Hernández-Serrano et al. (2013,2014), Budde et al. (2014), Calvo et al. (2016), Battersby et al. (2017), Table 3.

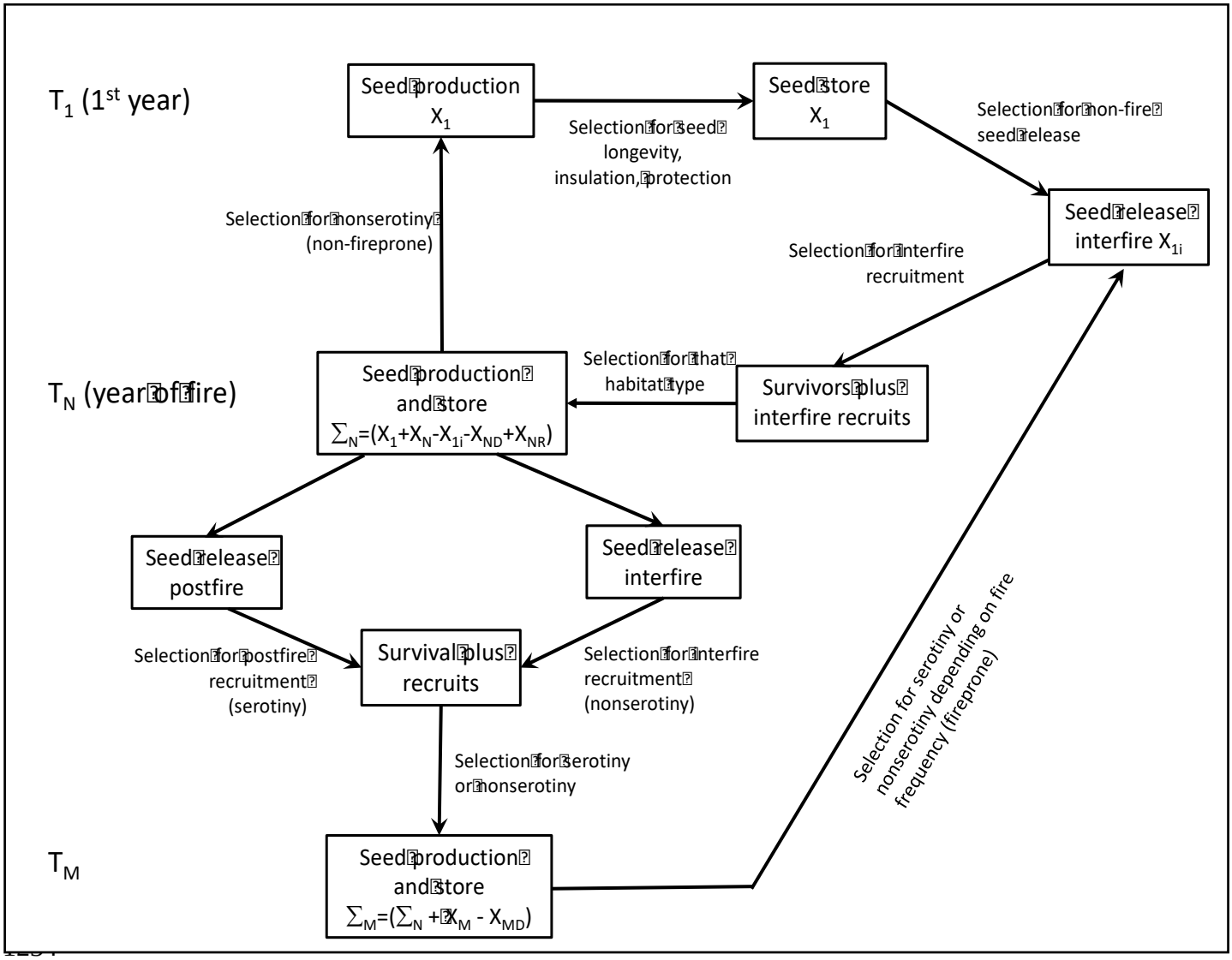


Fig. 7. Flow chart of the steps (boxed) and types of selection occurring between these stages (arrowed) involved in accounting for the evolution of particular levels of serotiny, or nonserotiny, at the population scale. The single interfire/fire cycle given here is repeated many times [T_M (year M) back to T_1] until an adaptive equilibrium is reached. X = number of seeds, i = interfire, Σ = total seed store, D = nonviable (dead) seeds, R = recruits. The fire occurs in year N – seed release interfire includes seeds released up to the time of the fire. Depending on their relative survival and fecundity in relation to the fire regime, strongly or weakly serotinous or nonserotinous individuals build up in the population. Summarized from Tables 1 and 2, and Figs 3, 5 and 6.

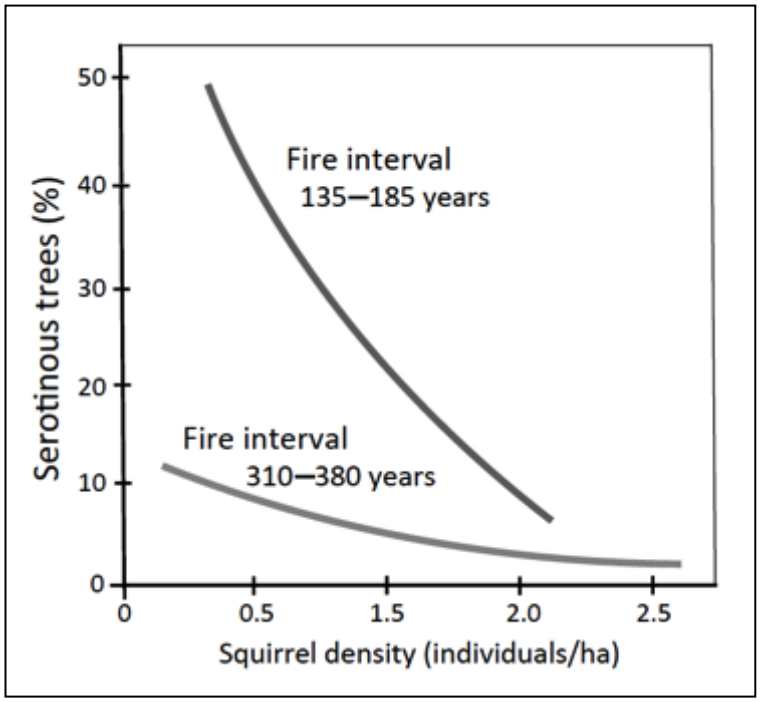


Fig. 8. Relationship between squirrel abundance (as an index of seed granivory) and level of serotiny (best fit lines to frequency of serotinous trees) among *Pinus contorta* subsp. *latifolia* stands at two mean fire intervals. Simplified and redrawn from Talluto and Benkman (2014).

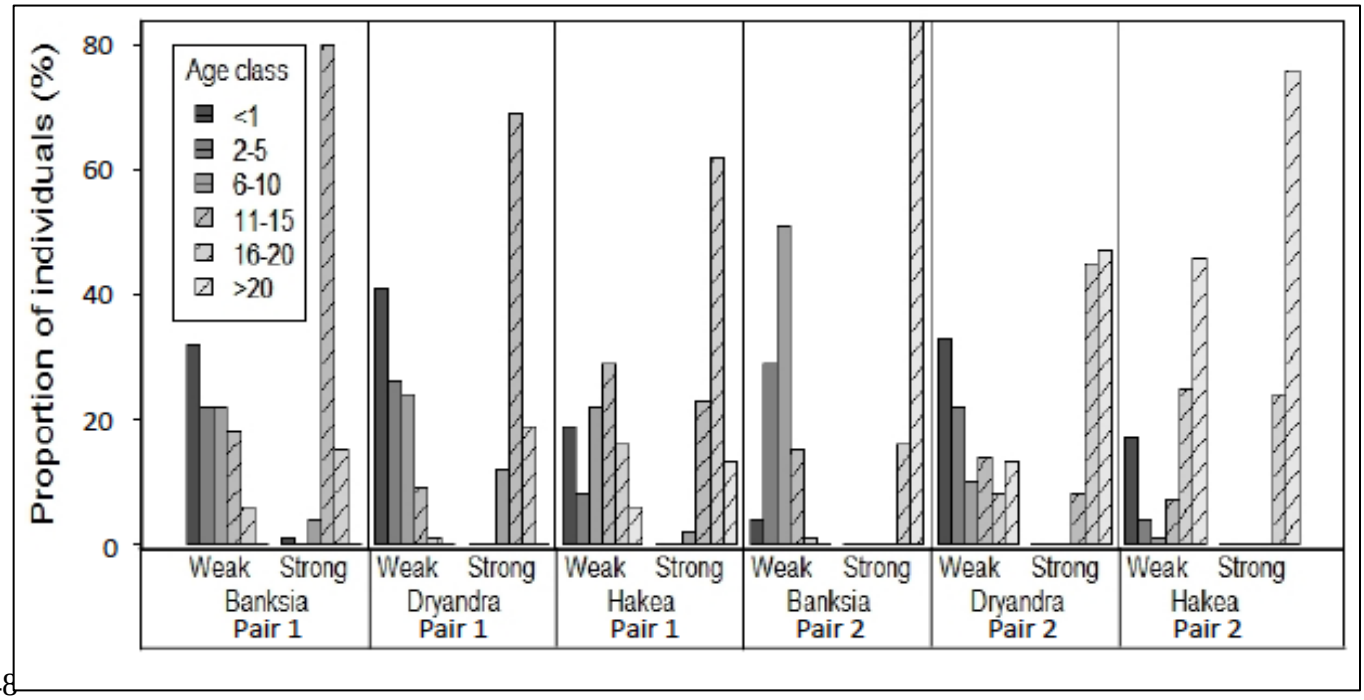


Fig. 9. Distribution of age classes among 12 species of woody shrubs in the Proteaceae (2 *Banksia*, 2 *Dryandra*, 2 *Hakea* species pairs) in scrub-heath vegetation of SW Australia. Within each genus, Pair 1 is separated from Pair 2 by 700 km and one of the pair is weakly serotinous (recruits spread over many years) and the other is strongly serotinous (recruits restricted to the year or so after fire). If a fire occurred at this time and assuming interfire plants < 10 years old make little contribution to seed availability (hatched bars), then 36% of weakly serotinous, prefire plants on average would contribute seeds to the next generation while 97% of strongly serotinous plants would. Drawn from data in Hanley and Lamont (2001).

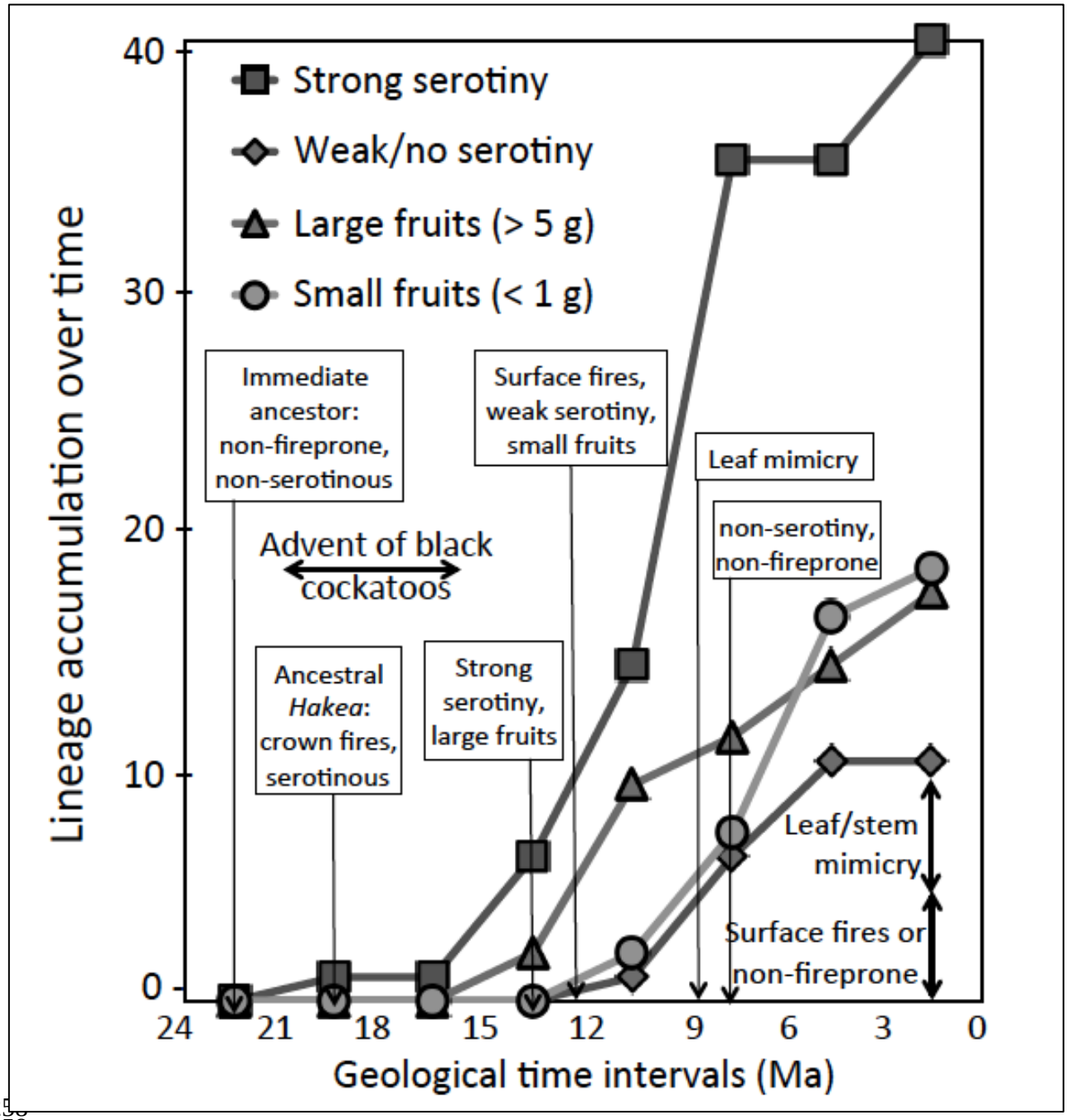


Fig. 10. Evolutionary history of serotiny and nonserotiny in the world's most sclerophyllous genus, *Hakea* (Proteaceae), in Australia based on ancestral trait assignment and molecular clock techniques (the subclade that possesses only strong serotiny has been omitted from this analysis). Arrows refer to the earliest evidence for the presence of certain traits or fire regimes. Note the few species with intermediate-sized serotinous fruits (1–5 g) are not plotted separately but are included in the total numbers under strong serotiny. Figure collated from Lamont et al. (2016a,b; 2017b).

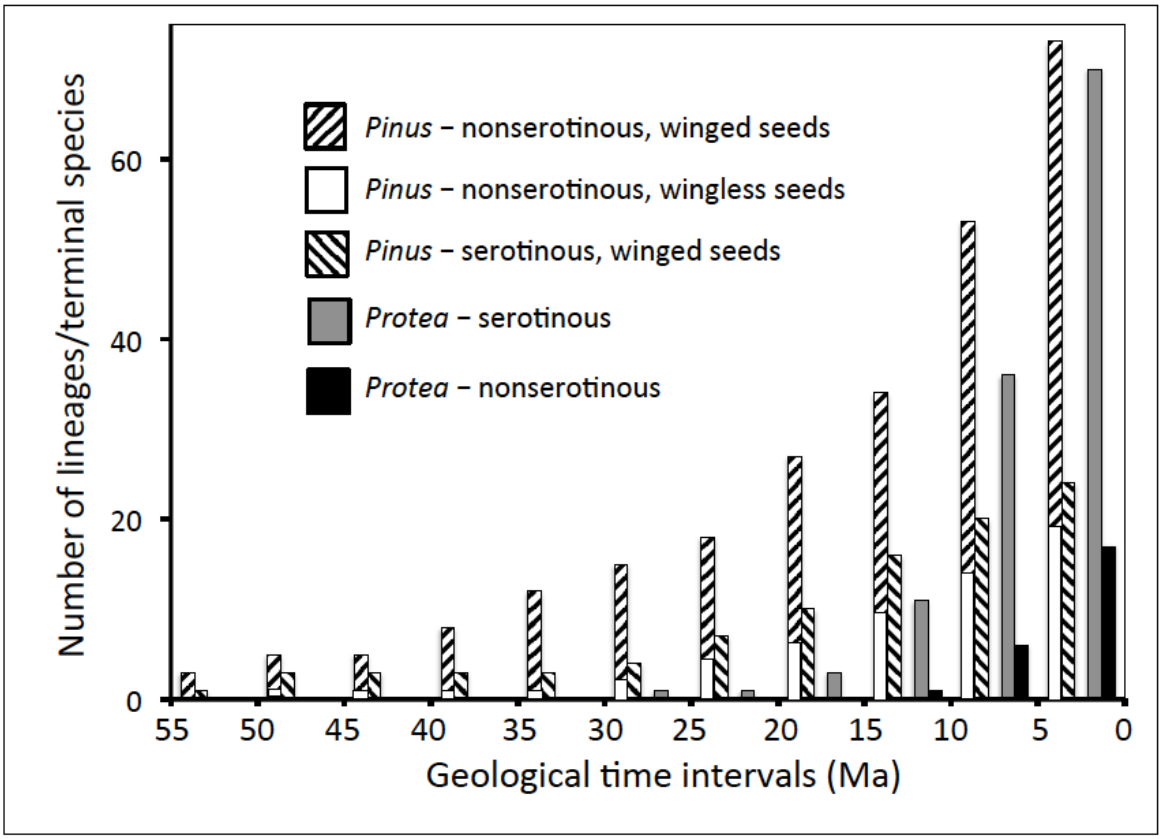
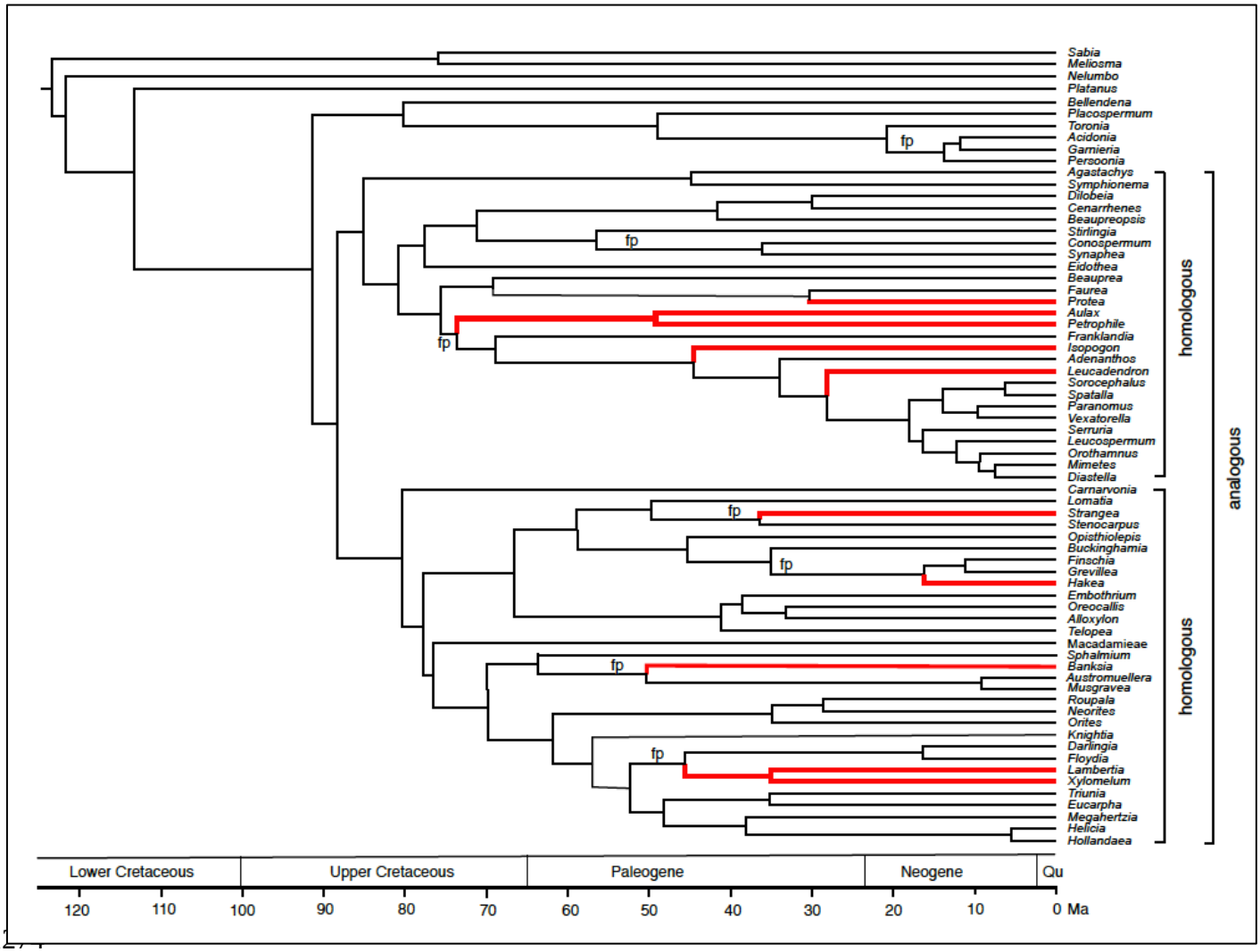


Fig. 11. Net total diversification of serotiny over geological time for *Pinus* and *Protea*, including the conversion of winged to wingless seeds among nonserotinous lineages in *Pinus* and loss of serotiny among resprouters in *Protea*. Data obtained from chronograms presented in He et al. (2012), Lamont, He and Downes (2013), Lamont, He and Pausas (2017a) and Lamont, He and Yan (2019b).



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1275 Fig. 12. Chronogram for the family Proteaceae showing the geological history of serotinous
1276 lineages (in red) vs non-serotinous lineages. Note how each of the 10 serotinous lineages
1277 commences in a fireprone (fp) environment but not all fp environments lead to serotiny.
1278 Homology exists at the fruit level within subfamilies but not between them (analogy, homoplasy).
1279 Adapted from Lamont and He (2017) and supplementary material in Lamont and He (2012).

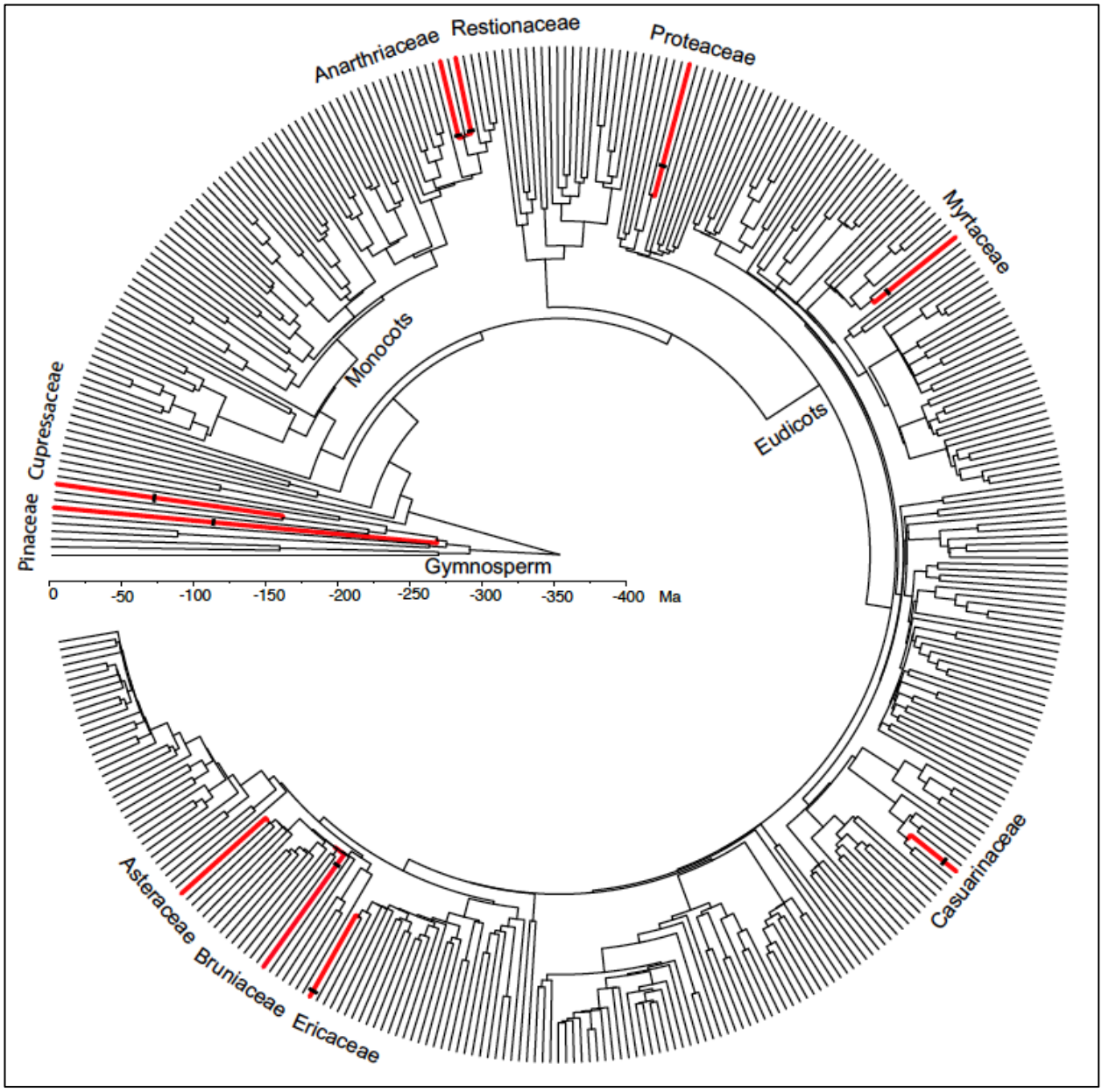

Fig. 13. Dated phylogeny for the world's seed-plant clades to which have been added lineages possessing serotinous species at the rank of family – in red. The approximate time that the lineage first displayed serotiny is indicated by a cross-bar. Note that in all cases the lineage would have been fireprone before, or at least coincident with, the onset of serotiny (Lamont and He 2017). *Sequioadendron* is embedded in Cupressaceae at about 20 Ma though it could be up to 45 Ma (Lowe, 2013/4). Backbone of the phylogeny was adapted from Zane et al. (2014).

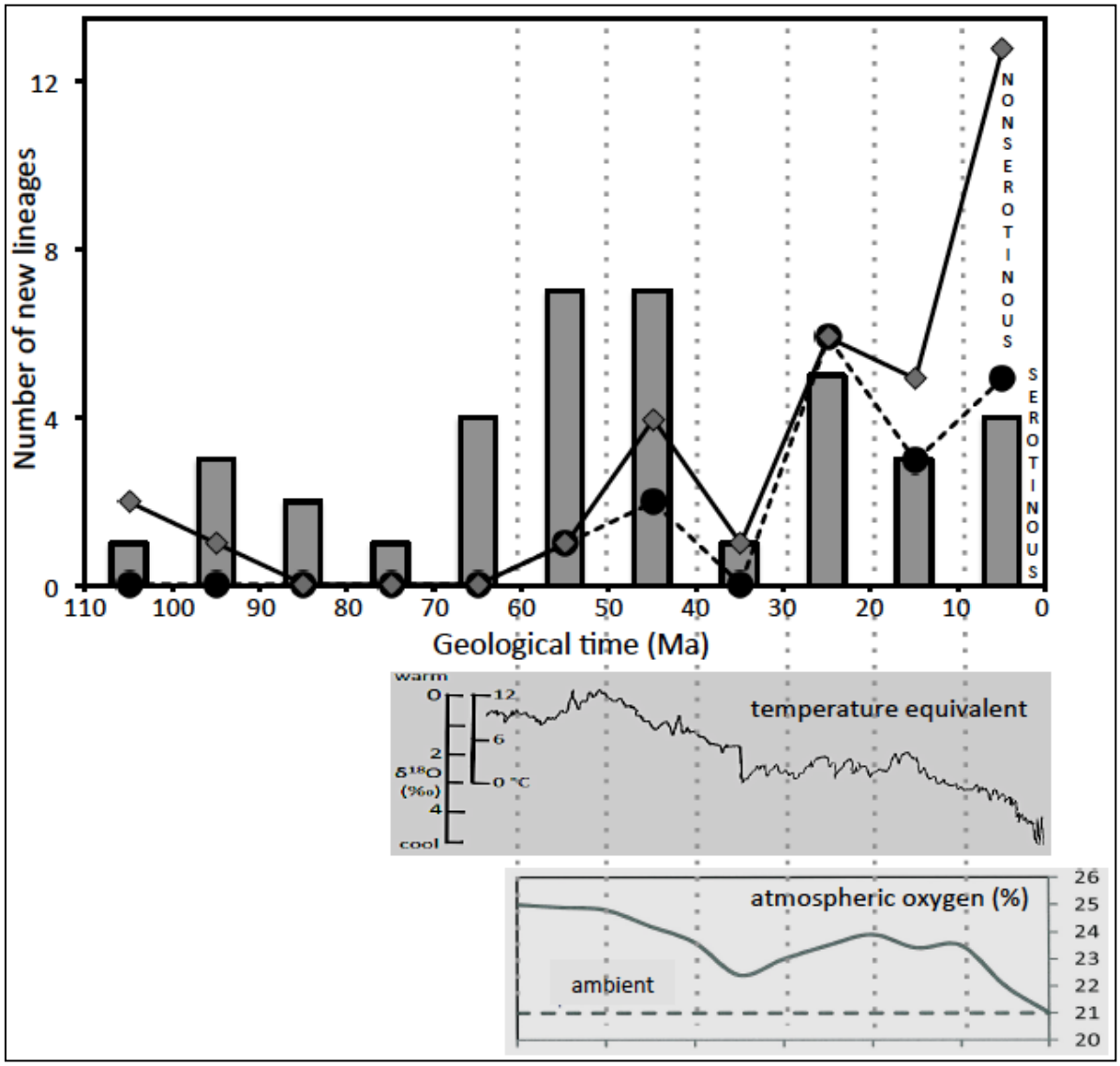

Fig. 14. Number of new lineages (bars) possessing serotinous cones/fruits (recorded using Bayesian probability trait reconstruction techniques) arising at 10 million-year (Ma) intervals since 110 Ma. Collated from data for 31 clades in the families Casuarinaceae, Cupressaceae, Myrtaceae, Pinaceae and Restionaceae in the Supplementary Material, Lamont and He (2017). Included are $\delta^{18}O$ values (as a surrogate for temperature, redrawn from Zachos, Dickens and Zeebe, 2008) and estimated atmospheric O_2 values (from Bergman, Lenton and Watson, 2004). Adapted from Lamont, He and Yan (2019a). Added are equivalent new data for the subfamily Callitroideae, Cupressaceae showing total new lineages (diamonds) and those with serotiny (filled circles) collated from supplementary data in Crisp et al. (2019). The difference between these two values is the number of nonserotinous lineages.

1299 **Table 1.** Traits within the serotiny syndrome (seed storage, pyriscence, postfire recruitment) or nonserotiny syndrome (seed release at maturity, interfire
1300 recruitment) and how these are affected by environmental and phylogenetic constraints, and whether the effect of the trait on each of the three
1301 components is positive or negative, direct or indirect, with generic examples and supporting references.
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Component (alternatives)	**Trait subject to selection**	**Environmental constraint**	**Phylogenetic constraint**	**Effect on component**	**Generic examples**	**References**
1. On-plant seed storage *vs* non-storage	Seed/fruit completely enclosed within a sealed supporting structure but has zone of weakness for enabling dehiscence	Growing conditions sufficient to ensure complete development of seed storage complex	Ability of surrounding structures to support and wrap around seed/fruit yet be dehiscent; propensity to produce enclosure sealants (resins) vs physical resistance limits of potentially flexing tissues	Direct	*Banksia*	Enright and Lamont, 1989; Huss et al., 2018, 2019
	Cone/fruit maintained in closed condition	Water and nutrient availability – rainfall, seasonal drought	Ability of vascular system to resist clogging, embolism	Direct	*Aulax*, *Hakea*, *Leucadendron*	Cramer and Midgley, 2009
	Decline in seed longevity matches rate of cone/fruit opening	Invasion by pathogens, granivores, rainwater, dry air	Propensity of enclosure to remain closed over time, inherent dormancy limits	Direct	*Eucalyptus*, *Melaleuca*, *Banksia*	Pannell and Myerscough, 1993; Lamont and Enright, 2000; Crawford et al., 2011; Fig. 1
	Insulation of seeds from heat (unrelated to fire)/cold – even if nonserotinous	Exposure to sunlight, hot/cold air currents, snow	Metabolic and anatomical properties of supporting structure	Indirect (insulation from the 'elements')	*Pinus*, *Banksia*, *Hakea*	Personal observations of fruits opening on sunny side of plant; see Table 3
	Fruit thick, woody, corky	Granivore pressure (ovipositing insects; parrots – cockatoos)	Metabolic and morphological properties of leaves (to supply carbohydrates) and supporting enclosure	Indirect (granivory)	*Hakea*	Groom and Lamont, 1997; Lamont et al., 2016a

	Cone scale complex thick, woody, spiny	Granivore pressure (crossbill corvids, squirrels)	Metabolic and morphological properties of leaves and supporting enclosure, propensity to develop spines	Indirect (granivory)	*Pinus*	Elliott, 1974; Moya et al., 2008
	Enclosure sealed off from atmosphere, water repellent	Exposure to water held in crevices, pathogens, dry air, air pollutants	Metabolic and anatomical properties of supporting enclosure	Indirect (waterlogging, pathogenesis, desiccation, pollutants)	*Banksia*	Lamont et al., 1991; Moya et al., 2008
	Accumulation by seed of nutrients vital for seedling recruitment in impoverished soils	Soil nutrient availability	Metabolic and vascular limits to supply nutrients to embryo over an extended period	Indirect*	*Hakea*	Lamont and Groom, 2013
	Propensity of cone to open at maturity (nonserotinous)	(Sub)tropical grassland/savanna, frequent fire, summer rainfall	Efficiency of dehiscence mechanism	Indirect (flame not reach crown)	*Banksia* (*dentata*), *Protea*, *Pinus*, *Larix*, *Hakea*	See Tables 4, 5, Fig. 6
	Propensity of cone to open at maturity	Habitat non-fireprone	Efficiency of dehiscence mechanism	Direct	*Banksia*, *Protea*, *Pinus*, *Hakea*	See Tables 4, 5
2. Pyriscence *vs* seed release at maturity	Fire-sourced heat able to stimulate operation of seed release mechanism directly or via death of supporting stem and thus supporting structure	Flammability of surrounding foliage and litter (fire of sufficient intensity)	Location of crown in relation to heat source (inherent plant growth rate), e.g., tree crown may escape heat from grass fires but prostrate plants will be burnt	Direct	*Banksia*, *Pinus*, *Eucalyptus*	Cowling and Lamont, 1985; Enright and Lamont, 1989; Habrouk, Retana and Espelta, 1999; Lamont and Enright, 2000, dos Santos et al. 2015

	Insulation of seed from fire heat	Intensity of fires	Insulation and non-flammability properties of supporting tissues	Direct	*Pinus*, *Banksia*, leptospermoid Myrtaceae	Judd, 1994; Habrouk, Retana and Espelta, 1999; Lovreglio et al 2007; Moya et al., 2008; Salvatore et al; 2010; Lamont et al., 1994b
	Tolerance of seed to fire heat	Intensity of fires	Heat-tolerant limits of dormant seed	Direct	*Calothamnus*, *Banksia*, *Hakea*, *Pinus*, *Dryandra*, *Allocasuarina*, *Eucalyptus*, *Melaleuca*	Habrouk, Retana and Espelta, 1999; Hanley and Lamont, 2000; Goubitz, Werger and Ne'eman, 2003; Lovreglio et al., 2007; Tangney et al., 2019
	High melting point of binding resins (opening mechanism)	Intensity of fires	Propensity for terpenoid synthesis	Direct	*Banksia*, *Pinus*	Enright and Lamont, 1989; Tapias et al., 2001
	Fruit/scale/bract reflexation (opening mechanism)	Intensity of fires	Tissue arrangement, secondary metabolism and anatomy relative to flexing requirements	Direct	*Banksia*, *Pinus*	Moya et al., 2008; Huss et al., 2018, 2019
	Central plate pulls seeds out of enclosure (seed release mechanism)	Intensity of fires	Propensity to produce a central plate (decipium) that can grasp seeds and reflex to pull seed out of fruit	Direct	*Banksia*, *Dryandra*	Cowling and Lamont, 1985b
	Wet-dry cycles required to ease seeds out of the supporting structure	Intensity of fires (redundant if flame temperature is high enough), postfire weather	Tissue arrangement, secondary metabolism and anatomy relative to flexing requirements	Direct	*Banksia*, *Dryandra*	Cowling and Lamont, 1985b; Lamont and Barker, 1988; Lamont and Enright, 2000

	Mantle of dead florets (high flammability)	Flame source	Ability to retard abscission layer development at base of florets	Direct	*Banksia*	Lamont and Cowling, 1984
	Dead leaf retention (high flammability)	Flame source	Ability to retard abscission layer development at base of leaves	Direct	*Banksia*	He, Lamont and Downes, 2011
	Dead branch retention (transports flames to crown)	Flame source	Ability to retain dead branches that serve to propagate flames	Direct	*Pinus*	Schwilk and Ackerly, 2001; He et al., 2012
	Heat not required to stimulate seed release mechanism (occurs at maturity or in response to other cues or gradual desiccation)	Interfire release (absence of fire)	Efficiency of dehiscence mechanism on reaching maturity	Direct	Species in otherwise serotinous genera can be nonserotinous	Lamont, He and Yan, 2019b
	Predispersal granivore avoidance	Interfire release (absence of fire)	Efficiency of dehiscence mechanism on reaching maturity	Direct	*Pinus*	Talluto and Benkman, 2013
3. Recruitment – postfire *vs* interfire	Heat-stimulated (predispersal) germination	Postfire release (fire occurrence)	Physiological limits of seeds to high-temperature tolerance	Direct	*Pinus*, *Allocasuarina*	Habrouk, Retana and Espelta, 1999; Hanley and Lamont, 2000; Lovreglio et al., 2007
	Postdispersal seed ash/charcoal/burial tolerance	Postfire release (fire occurrence)	Physiological/morphological limits of seeds re alkali/burial tolerance	Direct (minor)	*Banksia*, *Hakea*, *Pinus*, *Petrophile*	Lamont, Witkowski and Enright, 1993; Goubitz, Werger and Ne'eman, 2003
	Postdispersal seed tolerance of alkaline soil	Interfire release (absence of fire)	Physiological limits of seeds re alkali tolerance	Inverse (minor)	*Pinus*	Goubitz, Werger and Ne'eman, 2003
	Postdispersal seed high-temperature tolerance	Postfire release (fire occurrence)	Physiological limits of seeds re high temperature tolerance	Inverse (minor)	*Pinus*	Moya et al., 2013
	Postdispersal seed high-temperature tolerance	Interfire release (absence of fire)	Physiological limits of seeds re high temperature tolerance	Direct (minor)	*Pinus*	Moya et al., 2013

	Smoke-stimulated germination	Fire occurrence	If released interfire, ability to survive soil storage and fire required	Direct (minor)	*Leucadendron*	Brown and Botha, 2004
	Full-sun-tolerant seedlings	Postfire release (fire occurrence)	Drought/full-sun/heat tolerance of seedlings	Inverse	*Banksia*, *Hakea*, *Dryandra*	Causley et al., 2016
	Drought/shade-tolerant seedlings	Interfire release (absence of fire)	Drought/shade tolerance of seedlings, e.g., via large seeds	Direct	*Banksia*, *Dryandra*, *Hakea*, *Pinus*	Hanley and Lamont, 2001
	Postdispersal granivory minimization by synchronized seed release[#], mimicry	Postfire release (fire occurrence)	Limit to number and size of seeds that can be produced to satiate granivores	Direct	*Banksia*, *Pinus*	Lamont et al., 1991; Saracino et al., 1997
	Postdispersal herbivory minimization by synchronized seedling emergence[#]	Postfire release (fire occurrence)	Limit to number and size of seedlings that can be produced to satiate herbivores	Direct	*Banksia*	Lamont, Witkowski and Enright, 1993
	Strong heterospecific-competition tolerance	Postfire release (fire occurrence)	Limit to number and size of seedlings that can be produced	Direct	*Banksia*, *Hakea*, *Petrophile*	Lamont, Witkowski and Enright, 1993
	Strong conspecific-competition tolerance	Interfire release (absence of fire)	Limit to number and size of seedlings that can be produced	Direct	*Banksia*, *Dryandra*, *Hakea*	Hanley and Lamont, 2001

*Needs strong root system for recruitment

#Trait may be ineffective as burnt sites with abundant seeds and seedlings also attract granivores and herbivore

Table 2. Three scenarios of varying fire regimes and contrasting interfire/postfire conditions ,leading to three contrasting levels of serotiny through differential selection of the serotinous and nonserotinous phenotypes. See Fig. 7 for supporting references.

Fire-related trait	**Interfire events**	**Postfire events**	**Long-term outcome**
Strong serotiny (moderate fire frequency, poor interfire recruitment conditions)	Minor nonserotinous seed release, little seedling establishment, recruits rare and their fecundity low, massive serotinous seed buildup (nonserotinous phenotypes selected against as they do not contribute to the next generation)	Fire-caused death of at least some parents and interfire recruits, *en masse* release of serotinous seeds and seedling establishment, extensive seedling/juvenile death but many recruits still reach adulthood (fire promotes selection of serotinous phenotypes)	Buildup of increasingly serotinous adults with negligible interfire recruitment (of less serotinous adults), with increasingly even-aged stands (unless resprouts) (serotinous phenotypes much fitter than nonserotinous phenotypes)
Weak serotiny (low/highly variable fire frequency, interfire recruitment conditions almost as good as postfire)	Continuous release of nonserotinous seeds with weak buildup of serotinous seed store, pulses of recruitment, with different age classes contributing most to population size, many recruits reach maturity as vegetation gaps available for colonization and possess seeds at time of fire (serotinous phenotypes selected against as do not recruit interfire)	Fire-caused death of at least some parents and interfire recruits, *en masse* release of both seed types and seedling establishment, extensive seedling/ juvenile death but many recruits still reach adulthood, more than interfire (fire promotes selection of both phenotypes)	Buildup of both serotinous and nonserotinous adults with interfire recruits contributing substantially to successive generations with increasingly multi-aged stands (including resprouts) (serotinous phenotypes only marginally fitter than nonserotinous phenotypes)
No serotiny (high fire frequency, interfire recruitment conditions as good as, or better than, postfire)	Nonserotinous seed release, recruits rare but quickly attain resprouting capacity, insufficient time and resources for serotinous seed buildup (serotinous phenotypes selected against as cannot recruit interfire)	Fire survival of parents and older recruits, or survival in non-fireprone pockets, no more seeds released than annually interfire, recruitment no more likely than interfire if say a dry year (fire does not promote selection of either phenotype)	Buildup of nonserotinous, resprouting adults with interfire recruits contributing most to successive generations with increasingly multi-aged stands (nonserotinous phenotypes fitter than serotinous phenotypes)

Table 3. Mean effect of regional location (mesic vs xeric) and growing conditions (good vs poor) on plant size, fecundity, nutrient content, level of serotiny and fire-caused mortality among populations of two *Banksia* species in SW Australia and two *Pinus* species in Spain, both with Mediterranean-type climates. Statistical error terms are given in original papers. Note *Pinus pinaster* (on infertile siliceous substrates) and *P. halepens*is (mostly on more fertile calcareous substrates) are placed under poor and good growing conditions [sp(ecies) effect] to assist comparisons. NS: $P > 0.05$, **: P = 0.001–0.01, ***: P < 0.001. NS in bold highlights the lack of effect of growing conditions on the level of serotiny in contrast to regional effects. Data for banksias from Cowling and Lamont (1985a), Lamont et al. (1994a,b), Groom and Lamont (2011) and for pines from Hernández-Serrano et al. (2013), including their Table S2.

Species	**Attribute**	**Mesic sites, low crown fire frequency (surface fire)**		**Xeric sites, moderate crown fire frequency**		**Statistical tests**		
	Growing conditions	Poor	Good	Poor	Good	Site (S)	Growth (G)	S × G
Banksia menziesii	Length growing season (months)	6.2	>6.2	4.2	>4.2	-	-	-
	Height (m)	4.2	4.8	2.9	3.6	***	**	NS
	Crown size (m^3)	14.6	35.4	7.8	20.0	**	***	NS
	Shoot N (mg/g)	4.6	8.0	5.1	10.9	NS	*	NS
	Shoot P (mg/g)	0.2	0.5	0.3	0.6	NS	**	NS
	Shoot K (mg/g)	2.6	5.4	3.5	7.6	**	**	NS
	Seeds retained (%)	16.8	38.0	53.8	63.9	***	***	NS
	Seeds stored / plant	6.4	22.7	54.3	202.2	***	***	NS
	Serotiny (100/*b*)	6.6	5.6	9.4	8.3	******	**NS**	NS
	Fire-caused mortality (%)	5.5	-	21.3	-	**	-	-
Banksia hookeriana	Height (m)	-	-	1.54	2.02	-	***	-
	Crown size (m^3)	-	-	2.92	6.83	-	***	-
	Seeds stored / plant	-	-	952	3483	-	***	-
	Serotiny (100/*b*)	-	-	18.3	19.2	-	**NS**	-
	Fire-caused mortality (%)	-	-	100[#]	100[#]	-	-	-
Pinus pinaster (infertile soils), *P. halepensis* (fertile soils)	Mean annual temperature (°C)	12.4	13.0	14.7	15.3	-	-	-
	DBH (cm)	31.5	28.2	25.8	27.5	NS	NS	NS
	Closed cones (%)	11.3	26.5	32.6	45.5	***	*** (sp)	*
	Serotiny (max. age closed cones, y)	3.47	3.87	8.27	6.73	***	*** (sp)	***
	Fire-caused mortality (%)	?[$]	?[$]	100[#]	100[#]	-	-	-

[#] Observations at numerous sites

[$] Surface fires do not reach the crown to cause death

Table 4. Levels of serotiny associated with type of habitat-vegetation type, fire regime, relative interfire/postfire recruitment conditions and plant lifespan, with specific examples to show the wide variation that may occur even in the same genus. Supporting references: Heinselman (1981), Rebelo (2001), He, Lamont and Downes (2011), He et al. (2012), Lamont et al. (2017b), Lamont, He and Yan (2019b).

Habitat–vegetation type	Fire frequency (relative to plant lifespan)	Fire type	Interfire (I) recruitment conditions*	Postfire recruitment conditions	Plant lifespan	Serotiny	Examples
Rainforest, rock outcrop, desert, deciduous forest, wetlands, alpine	■ nil	not apply	variable	not apply	variable	■ nil	*Hakea clavata, Banksia integrifolia, Protea glabra, Pinus edulis, Larix decidua*
Wet/boreal/mixed forest, wetland margins, subalpine	■■ low	crown	good	good (> I)	long	■■ weak	*H. lasiantha, B. littoralis, Protea rubropilosa, Pinus serotina, L. gemelinii*
Dry forest, woodland, scrub, heath	■■■ low–moderate	crown	poor	good	variable	■■■ strong	*H. cucullata, B. serrata, Protea neriifolia, Pinus contorta*
Woodland, scrub, heath, savanna	■■■■ moderate	crown–surface	good	good (> I)	limited (not dominants)	■■ weak	*H. trifurcata, B. prionotes, Protea scabra, Pinus halepensis*
Savanna, (sub)tropical grassland with subshrub geoxyles	■■■■■ high	surface	variable	variable	long	■ nil	*H. lorea, B. dentata, Protea caffra, Pinus cubensis, L. occidentalis*

The squares indicate in a semiquantitative way how the levels of serotiny rise and fall with increasing fire frequency.

*as perceived by the species (see Fi

Table 5. Number of serotinous species/total number of species in relation to fire response and fire regime, for five clades: *Pinus* (Pinaceae; He et al. 2012), *Banksia* (Proteaceae; He et al., 2011), Callitroideae (Cupressaceae; Ladd et al., 2013; Crisp et al., 2019), *Protea* (Proteaceae; Lamont et al., 2013, 2017a), and *Hakea* (Lamont et al., 2017b; Fig. 5). Clades are listed in increasing time since their origin of serotiny (in brackets).

Clade	Fire response	Fire regime		
		No fire	Surface fire	Crown fire
Pinus (113 Ma)	Fire-killed	0/165	0/0	17/17
	Fire-survivor	0/0	0/11	11/11
Banksia (62 Ma)	Fire-killed	0/0	0/0	43/45
	Fire-survivor	0/0	0/1	33/39
Callitroideae (58, 48 Ma)	Fire-killed	0/14	0/0	17/17
	Fire-survivor	0/0	0/3	2/2
Protea (28 Ma)	Fire-killed	0/0	0/0	51/51
	Fire-survivor	0/0	0/23	20/22
Hakea (20 Ma)	Fire-killed	0/0	0/2	41/44
	Fire-survivor	0/1*	0/2	31/33
Overall	Fire-killed	0/179	0/2	169/175
	Fire-survivor	0/1*	0/40	97/107

*Lignotuberous

Supplementary Material

Table S1. Genera with serotinous seeds/fruits, including their geographic distribution, morphology of serotinous structures and seeds, number of serotinous species of those examined and total in genus, their habitat and that of any species lacking serotiny, and supporting references. We accept the view of *Udovicic and Spencer* (2012) on the taxonomy of Melaleuceae. Weak serotiny: at least some seeds held 1–4 y, moderate: 5–9 y, strong: 10+ y. Observations build on those given in Table 1 of Lamont et al. (1991) with new genera indicated by *. NA = not apply.

Family/ subfamily	**Genus**	**Distribution**	**Species serotinous/ examined/total in genus**	**Serotinous structure (all release seeds/fruits in response to fire and, to a lesser extent, the passage of time)**	**Dispersal unit, non-dormant unless indicated**	**Habitat serotinous (all fireprone)**	**Non-serotinous species**	**Habitat non-serotinous (variably fireprone)**	**References**
Cupressaceae ss	*Cupressus* ss	N Hemisphere	4/25 /25	Globular cone, 8–40 mm diameter, often warty with 4 scale complexes, and several seeds per scale	Weakly winged seeds (cones dehiscent)	Dry mountain forests	Yes,	Temperate, boreal forests or alpine uplands (non-fireprone)	Dallimore and Jackson 1966, Crisp et al. 2019
Cupressaceae ss	*Callitris* ss	Oceania, essentially Australia marginally to N Caledonia	13/16/16	Globular cone, 10–30 mm diameter, modertaly serotinous	Weakly winged seeds (cones dehiscent)	Sclerophyll heath to thickets of *Callitris* small trees	Yes	Grassland savannas, desert	Crisp et al. 2019
Cupressaceae ss	*Actinostrobus*	SW Australia	3/3/3	Pyramidal cone, 15 mm long, with scale complexes and extra bracts, and a few seeds per scale, moderately serotinous	Weakly winged seeds (cones dehiscent)		No	NA	Crisp et al. 2019
Cupressaceae ss	*Widdringtonia*	S Africa	3/4/4	Globular cone, 20–30 mm diameter, moderately serotinous	Weakly winged seeds (cones dehiscent)	fynbos, grasslands	Yes	woodlands, grasslands, fire-protected microsites	Crisp et al. 2019
Cupressaceae ss	*Tetraclinis*	W Mediterranean Basin	1/1/1	Globular cone with 4 scale complexes, weakly serotinous	Weakly winged seeds (cones dehiscent)	Scleophyll shrubland	No	NA	Dallimore and Jackson 1966
Pinaceae	*Pinus*	N Hemisphere	24/115/115?	Conic to ovoid cone, 30–600 mm long, with scores of scale complexes arranged in a spiral pattern decreasing in size towards the tip of the	Strongly apically winged seeds (cones dehiscent)	Mediterranean shrublands to forests,	Yes	Temperate, boreal forests or alpine uplands (non-	Table 6, He et al. 2012

				cone, sometimes with sharp appendages attached to the apophyses, weakly to strongly serotinous (the oldest recorded may be partly embedded in supporting branch)		temperate to subtropical woodlands and forests		fireprone) to or savanna woodlands (fireprone)	
Pinaceae	*Picea*	N America, N Europe	1/32/32	Pine-like, ovoid to cylindrical cone with spiralling loose scale complexes, weakly serotinous	Winged seeds (cones dehiscent)	Boreal forests	Yes (most)	Temperate, boreal forests	Safford 1974, He et al. 2012
Pinaceae	*Larix*	Temperate-cold zones of N Hemisphere	1/10/11	Cone with loose, petal-like, chartaceous scales varying greatly in size, with many seeds though often sterile, weakly serotinous. *L. gmelinii* is the only species to retain its cones after maturation	Winged seeds (cones dehiscent)	Temperate uplands to northern boreal lowlands	Yes (most)	Temperate, boreal forests or savanna woodlands	Table 3, He et al. 2012
Taxodiaceae ss	*Sequoiadendron*	California	1/1/1	Small, pine-like cone with thick apophyses, strongly serotinous (up to 20 years)	Lateral winged seeds (cones dehiscent)	Tall redwood forest in mountains	No	NA	https://en.wikipedia.org/wiki/Sequoiadendron_giganteum (6 Jan 2020)
Proteaceae/Grevilleoideae	*Banksia ss*	Australia, marginally to Papua New Guinea	76/86/86	Scattered, woody, rounded follicles, bearing two winged seeds and central winged plate, surrounded by mantle of fibrous bracts/bracteoles/(and sometimes persistent florets) attached to rachis to form a 'cone' (Fig. 1), weakly to strongly serotinous	Apically winged seeds (dehiscent fruits)	Sclerophyll low heath to forest	Yes (rare, nonstored)	Wetland, rock outcrop, savanna	George 1981, He et al. 2011
Proteaceae/Grevilleoideae	*Banksia* series *Dryandra*	SW Australia	48/50/94	Capitulum of thin, woody follicles, bearing two seeds and central winged plate, involucral bracts at base (and sometimes terminal foliage) wrap around fruits and are burnt off by fire, florets and bracts often deciduous, persistent style rarely modified into spine (*B. mimica*), weakly to strongly serotinous	Apically winged seeds (dehiscent fruits)	Sclerophyll low heath to forest	Yes (rare, nonstored)	Sclerophyll forest	B. Lamont, pers. observ.
Proteaceae/Grevilleoideae	*Hakea*	Australia	92/102/172	Solitary, or sometimes loosely clustered, extremely woody, axillary follicles bearing two winged seeds, classifiable into three groups: cryptic fruits < 1 g within spiny foliage, tend to mimic leaves/stems, remain green and be weakly serotinous, and exposed fruits > 5 g on	Apically, rarely annular, winged seeds (dehiscent fruits)	Sclerophyll low heath to forest	Yes (rare, nonstored)	Wetland, rock outcrop, saline soils, savanna grasslands, sparsely vegetated desert sands	Lamont et al. 2016a,b, 2017b, P.K. Groom, pers. comm.

				stout stems that resist bird granivory, turn grey/brown and be strongly serotinous, or are intermediate between these two extremes (Fig. 1)					
Proteaceae/Grevilleoideae	*Strangea*	SW, E Australia	3/3/3	Solitary semiwoody follicle supporting one winged seed, weakly serotinous	Annular or bi-apically winged seeds (dehiscent fruits)	Sclerophyll wet/dry heath to open forest	No	NA	Hnatiuk 1995a
Proteaceae/Grevilleoideae	*Lambertia*	SW, E Australia	10/10/10	Solitary, woody, thin follicle supporting two flat seeds fitting into similar categories as for *Hakea* except all fruits < 1 g and tend to be cryptic, most are highly ornamented (Fig. 1), weakly serotinous	Narrowly winged or wingless flat seeds (dehiscent fruits)	Sclerophyll heath (mainly), mallee to forest	No	NA	Hnatiuk 1995b
Proteaceae/Grevilleoideae	*Xylomelum*	E, SW Australia	6/6/6	Solitary, extremely woody, pear-shaped follicle, 60–90 mm long, with velvety surface, bearing two winged seeds (Fig. 1), strongly serotinous	Apically winged seeds (dehiscent fruits)	Scrub-heath to dry sclerophyll forest	No	NA	Foreman 1995a
Proteaceae/Grevilleoideae	*Telopea**	SE Australia	1/5/5	Scattered, elongated, leathery follicles, 1–8, containing up to 20 winged seeds, some of which may persist in open follicles into the second year	Apically winged seeds (dehiscent fruits)	Sclerophyll forest	Yes (all)	Heath to temperate rainforest	Crisp and Weston 1995, pers. observ.
Proteaceae/Proteoideae	*Protea*	Africa, tropica l to temperate	77/112/112	Capitulum of spindle-shaped achenes with persistent florets supported by tightly or loosely wrapped involucre of bracts (Fig. 1), weakly to moderately serotinous	Hairy-based achenes with persistent style sometimes burnt off (indehiscent fruits)	Sclerophyll low heath to tall shrubland	Yes (derived, widespread lineage, nonstored)	Savanna, (sub)tropical grassland	Lamont et al. 2013
Proteaceae/Proteoideae	*Aulax*	S Africa	3/3/3	Semiwoody cupule with reduced racemes (variously empty, leaf-like bracteoles on rachises of vestigial cones) around a short central rachis (cone) supporting four or more achenes subtended by bracteoles	Hairy-based achenes with persistent style (indehiscent fruits)	Sclerophyll heath	No	NA	Rourke 1998, Lamont and He 2012

Proteaceae/Proteoideae	*Leucadendron*	S Africa	44/81/82	Solitary terminal cone of tightly packed scales (bracteoles) many subtending achenes, usually surrounded by a loose involucre of conspicuous, colorful bracts, weakly to moderately serotinous	Achenes either variously winged or wingless nutlets rarely retaining the parachute-like perianth (indehiscent)	Sclerophyll low heath to tall shrubland with emergent trees (*L. argenteum*)	Yes (common, soil-stored nutlets)	Sclerophyll low heath to tall shrubland	Williams 1972, Tonnabel et al. 2017,
Proteaceae/Proteoideae	*Petrophile*	Australia, mostly SWA	53/53/53	Solitary, rarely clustered, terminal or axillary cones of woody scales (bracteoles) each supporting compressed nuts with conspicuous tufts of hairs or wings (or sterile) wrapped loosely around a rachis, sometimes with involucral bracts at base (Fig. 1), weakly to moderately serotinous	Hairy or winged nuts (indehiscent fruits)	Sclerophyll low heath to forest	No	NA	Foreman 1995b
Proteaceae/Proteoideae	*Isopogon*	Australia, mostly SWA	35/35/35	Solitary, rarely clustered, terminal cones (drumsticks) of multiple spiralling woody scales (bracteoles) each supporting nuts with conspicuous tufts of hairs (or sterile) wrapped tightly around a rachis (Fig. 1), weakly to moderately serotinous	Hairy nuts (indehiscent fruits)	Sclerophyll low heath, wet/dry scrub-heath to mallee to forest	No	NA	Foreman 1995c, Pausas and Lamont 2018
Proteaceae/Proteoideae	*Conospermum**	Australia, mostly SWA	2/53/53	Compound infructescence with swollen, moist branched peduncle whose tips engulf ovoid fruits in groups of three, moderately serotinous	Fruits with ring of hairs (indehiscent fruits)	Sclerophyll low heath to forest	Yes (most, geosporous)	Sclerophyll low heath to forest	Zhao and Ladd 2015
Casuarinaceae	*Allocasuarina*	Australia, half SWA	26/27/49 (E Australian spp not examined)	Solitary, woody, globular/cylindrical cones on short axillary peduncles, loosely clustered along branches, each bearing tightly clustered pairs of multiple spiralling valves (bracteoles, sometimes with sharp apices or appendages), subtended by an inconspicuous bract, almost all supporting single samaras, weakly to strongly serotinous (Fig. 1)	Samaras with hyaline, apical wings with midribs (indehiscent fruits)	Sclerophyll low heath to forest, sometimes forming thickets	No	NA	Grieve 1988, Wilson and Johnson 1989, Paczkowska and Chapman 2000
Lyginiaceae (Restionaceae)	*Lyginia**	SW Australia	3/3/3	Terminal, compressed globose, woody, trilocular capsule with persistent style, surrounded by chartaceous bracts, laterally	Wingless rounded seed with spinules	Sclerophyll low heath to	No	NA	Meney and Pate 1999a, Briggs and Johnson 2000

				dehiscent with one seed per chamber, weakly serotinous	and medial flange (dehiscent fruits)	Banksia woodland			
Anarthriaceae (Restionaceae)	*Anarthria**	SW Australia	7/7/7	Terminal or subterminal, globose, woody, trilocular capsule with persistent styles, surrounded by persistent chartaceous perianth, laterally dehiscent with one seed per chamber, weakly serotinous	Wingless rounded seed, < 1 mm diameter (dehiscent)	Wet/dry heath to sclerophyll woodland	No	NA	Meney and Pate 1999b, Briggs and Johnson 2000
Restionaceae	*Askidosperma**	S Africa	1/1/	weakly serotinous	Nut, germination benefits from smoke	Sclerophyll low heath to scrub-heath	?		Brown, Jamieson and Botha 1994
Restionaceae	*Cannomois**	S Africa	2/2/12	Short spike with six of so chitinous bracteoles subtended by inconspicuous bracts enclosing a single nut, weakly serotinous (Fig. 1)	Hard nut with aril (eliaosome), germination may benefit from smoke	Sclerophyll low heath to scrub-heath	?		Brown, Jamieson and Botha 1994
Restionaceae	*Hypodiscus**	S Africa	2/2	weakly serotinous	Nut, germination requirements unknown	Sclerophyll low heath to scrub-heath	?		Brown, Jamieson and Botha 1994
Restionaceae	*Willdenowia**	S Africa	1/1	weakly serotinous	Nut, germination requirements unknown	Sclerophyll low heath to scrub-heath	?		Brown, Jamieson and Botha 1994
Myrtaceae/ Leptospermoideae	*Eucalyptus* ss	Australia, marginally to New Guinea and SE Asia	399/403/~671 (SWA, Yalgoo, Coolgardie + few N-Central spp examined)	Solitary (large) to umbels with 3 to many (semi)woody globose, cup- to urn-shaped capsules, sessile (globular cluster) or pedicellate, with inserted or exserted valves and often ribbed, warty or other ornamentations on the hypanthial cup, with each of 3–8 chambers bearing 1 or more fertile seeds and many aborted seeds (Fig. 1), weakly to moderately serotinous	Angular to ellipsoid winged seeds (dehiscent fruits)	Sclerophyll heath to mallee to tall closed forest	Yes, poorly known (nonstored)	Grassy savannas, isolated on bare uplands – single trunk with remote crown	Grieve 1980a (excluding *Corymbia*, including hybrids), Paczkowska and Chapman 2000, Euclid 2006
Myrtaceae /Leptospermoidea e	*Corymbia* (*Eucalyptus* sl)*	Australia	7/14/~113 (SWA, Yalgoo, Coolgardie + few N-Central spp examined)	Clusters of urn-shaped, woody capsules with valves inserted below a distinct rim, with pedicels of varying lengths to form a flat-faced corymb, weakly to moderately serotinous	Flat, wingless, angular seeds (dehiscent fruits)	Sclerophyll woodland to tall forest	Yes, poorly recorded, common in N-	Grassy savannas, isolated on bare uplands – single trunk with remote crown	Grieve 1980a (*Corymbia* sunk in *Eucalyptus*), Paczkowska and

							Central Australia, Fig. 1 (nonstored)		Chapman 2000, Euclid 2006
Myrtaceae/ Leptospermoideae	*Angophora (Eucalyptus* sl)*	E Australia	3/6/16	(Compound) umbels of wineglass-shaped, semiwoody capsules with persistent sepals, ribbed hypanthium and sometimes hispid indumentum, weakly serotinous	Flat, to ellipsoid winged seeds (dehiscent fruits)	mallee to sclerophyll forest	Yes, poorly recorded but appears common (nonstored)	Sclerophyll woodland to forest	Chippendale, 1988, Euclid 2006
Myrtaceae/ Leptospermoideae	*Melaleuca*ss (excluding all other Melaleuceae listed here)	Australia, marginally to Lord Howe Isl, New Caledonia and Asia, as far W as Myanmar	177/177/~251 (only SWA spp + Yalgoo, Coolgardie regions examined)	Loose, cylindrical to tight, globular clusters of a few to scores of woody, cup- to urn-shaped capsules ~5 mm long, at first axillary, terminal or subterminal (rarely cauliflorous) then located at intervals along the bare supporting branches, hypanthium smooth rarely ribbed, valves inserted, 3-5 chambers with many seeds (Fig. 1), weakly to strongly serotinous	Tiny, elongated, angular seeds (dehiscent fruits) (Fig. 1)	Sclerophyll wet/dry heath to forest, often dominant small trees fringing water bodies	? (poorly recorded but must be common in N Australia, nonstored)	? Grassy savannas in N Australia	Grieve 1980b, Paczkowska and Chapman 2000
Myrtaceae/ Leptospermoideae	*Agonis*, includes *Taxandria* and *Paragonis*	SW Australia	12/12/16	Axillary, semiglobular clusters of < 10 woody capsules, < 10 mm long, with deciduous bracteoles and 2–5 chambers with inserted valves, weakly serotinous	Wingless, angular seeds (dehiscent fruits)	Fringing swamp vegetation, scrub-heath to forest	No	NA	Grieve 1980c, Paczkowska and Chapman 2000; Wheeler and Marchant 2007
Myrtaceae/ Leptospermoideae	*Beaufortia*	SW Australia	20/20/20	Tight, globular clusters of < 20 woody, cup-shaped capsules ~5 mm long, initially terminal or subterminal then located along the supporting woody branches, weakly to moderately serotinous	Wingless, angular seeds (dehiscent fruits)	Wet/dry low heath to scrub-heath	No	NA	Grieve 1980d, Paczkowska and Chapman 2000
Myrtaceae/ Leptospermoideae	*Callistemon*	Australia, mainly E temperate,	50/51/51	Tight, cylindrical clusters of woody, cup-shaped capsules ~5 mm long, initially subterminal then located at intervals along the bare supporting	Tiny, elongated, angular seeds	Fringing swamp vegetation,	Possibly *C. nervosus*	Lignotuberous mallee-like in grassland savanna	Grieve 1980e, https://en.wikipedi

		marginally to N Caledonia		branches (Fig. 1), moderately to strongly serotinous	(dehiscent fruits) (Fig. 1)	scrub-heath to forest		with interfire recruits evident	a.org/wiki/Callistemon (4 Jan 2020)
Myrtaceae/ Leptospermoideae	*Kunzea*	Mainly SW Australia, marginally NZ	22/24/25	Subterminal, or terminal on short axillary branchlets, globular clusters of <10, semiwoody capsules, with thin deciduous bracts and bracteoles, sepals sometimes persistent, 2–5 chambers with numerous small seeds, weakly or nonserotinous	Tiny, wingless angular seeds (dehiscent fruits)	Fringing wetland vegetation to rock outcrops, scrub-heath	Yes	Non-fireprone vegetation in NZ	Burrell 1965, Grieve 1980f, Paczkowska and Chapman 2000
Myrtaceae/ Leptospermoideae	*Calothamnus*	SW Australia	45/45/45	Globose to cup-shaped capsules crowded in loose clusters on one side of branch at intervals, sometimes partly embedded in branches, with inserted valves, inflexed woody sepals, smooth or warty hypanthium, 3–5 chambers with numerous small seeds, moderately serotinous	Wingless, rounded seeds (dehiscent fruits)	Sclerophyll low heath to open forest	No	NA	Grieve 1980g, Paczkowska and Chapman 2000
Myrtaceae/ Leptospermoideae	*Leptospermum*	Australia, marginally to SE Asia, New Zealand	18/18/86 (only SWA ad NZ spp examined)	Solitary, semiwoody to woody, cup-shaped capsules, < 10 mm long, with 3–10 chambers with inserted valves and bearing numerous small seeds, *L. spinescens* corky (Fig. 1), weakly to moderately serotinous	Tiny, elongated, wingless, angular seeds (dehiscent fruits)	swamp vegetation sometimes forming thickets, scrub-heath to forest	Yes (nonserotinous spp and populations are known)	Alpine and other non-fireprone vegetation	Grieve 1980h, Paczkowska and Chapman 2000, Battersby et al. 2017
Myrtaceae/ Leptospermoideae	*Conothamnus*	SW Australia	3/3/3	Globular clusters of globose capsules < 5 mm long on bare branches, with remnants of involucral bracts in *C. trinervis*, weakly serotinous	Tiny, wingless, angular seeds (dehiscent fruits)	Wet/dry low heath to scrub-heath	No	NA	Grieve 1980i, Paczkowska and Chapman 2000
Myrtaceae/ Leptospermoideae	*Eremaea*	SW Australia	19/19/19	Solitary, woody, globose to cup-shaped capsules, < 10 mm long, sessile on old stems, sepals rarely retained, with 3–10 chambers with inserted or humped valves, and bearing numerous small seeds, weakly to moderately serotinous	Tiny, wingless, angular seeds (dehiscent fruits)	Sclerophyllous low heath to woodland	No	NA	Grieve 1980j, Paczkowska and Chapman 2000
Myrtaceae/ Leptospermoideae	*Phymatocarpus*	SW Australia	2/2/2	Globular to cylindrical clusters of < 15, globose capsules on bare branches with inserted valves, weakly to moderately serotinous	Tiny, wingless, angular seeds (dehiscent fruits)	Wet/dry heath	No	NA	Grieve 1980k, Paczkowska and Chapman 2000

Myrtaceae/ Leptospermoideae	*Regelia*	SW Australia	5/5/5	Globular clusters of 3–20, globose to cup-shaped capsules with inserted valves to three chambers with many seeds, weakly to moderately serotinous	Tiny, wingless, angular seeds (dehiscent fruits)	Scrub-heath	No	NA	Grieve 1980L, Paczkowska and Chapman 2000
Myrtaceae/ Leptospermoideae	*Lamarchea**	SW Australia	2/2/2	Solitary, axillary, globular, woody capsule with inserted valves to two chambers with numerous seeds, weakly to moderately serotinous	Wingless, angular seeds (dehiscent fruits)	Emergent in sclerophyll scrub-heath	No	NA	Grieve 1980m, Paczkowska and Chapman 2000
Myrtaceae/ Leptospermoideae	*Tristania* (different sp assessed in Lamont et al. 1991)	E Australia	0/1/1	(Umbels of leathery capsules similar in form to *Angophora*)	Wingless, angular, linear seeds (dehiscent fruits)	NA	Yes	Edge of waterways in sclerophyll forest	http://www.flickr.com/photos/tony_rodd/549359766
Myrtaceae/ Leptospermoideae	*Lophostemon**	E Australia, marginally to New Guinea	1/4/4	(Compound) umbels of wineglass-shaped, semiwoody capsules with deciduous sepals, weakly serotinous	Wingless, angular, linear seeds (dehiscent fruits)	Sclerophyll woodland to tall forest	Yes	Rainforest margins to sclerophyll forest	Wilson and Waterhouse 1982
Myrtaceae/ Leptospermoideae	*Xanthostemon*	N Australia to Malesia, mostly New Caledonia	0/24/51	(Cymes of semiwoody, globose capsules with persistent calyx)	Flat to angular, orbicular seeds (dehiscent fruits)	Savanna woodland	Yes (almost all)	Rainforest and creek margins to sclerophyll forest	Wilson 1990, B. Lamont, pers. observ. in New Caledonia
Myrtaceae/ Leptospermoideae	*Syncarpia**	E Australia	2/2/3	Globular woody capsules fused at base to form compound fruit with persistent calyx and inserted valves with many seeds, weakly serotinous	Linear, angular seeds (dehiscent fruits)	Sclerophyll forest	?	Non-fireprone forest?	Bean 1995
Myrtaceae/ Leptospermoideae	*Tristaniopsis*	Australia, mostly SE Asia, New Caledonia	0/2/30	No serotinous species located	NA	NA	Yes (all?)	Edge of waterways in sclerophyll forest to rainforest	Wilson and Waterhouse 1982
Asteraceae	*Syncarphass** (*Helipterum*)	Cape, S Africa	2/15/21	Capitulum of many cypselas with pappuses held in place by papery involucral bracts wrapped around them, reflexing on death of plant, usually from fire, weakly serotinous	Cypsela fruits with a pappus of bristles varying from smooth to plumose and fused basally into a ring	Sclerophyll heath	yes	Sclerophyll heath	Bond 1985, Bergh, Haiden and Verboom 2015

Asteraceae	*Phaenocoma*	Cape, S Africa	1/1/1	Capitulum of many cypselas with pappuses held in place by papery involucral bracts wrapped around them, reflexing on death of plant, usually from fire, weakly serotinous	Cypsela fruits with a pappus	Sclerophyll low heath	No	NA	Bond 1985
Bruniacaeae	*Berzelia*	Cape, S Africa	13/13/16	Subglobular, few-fruited raceme of dry nutlets with red, fleshy sepals in some species to form a compound structure of berry-like fruits that gradually dry out to release nutlets	Single-seeded dry nutlets (indehiscent)	Sclerophyll low heath to scrub-heath	No?	?	Lamont et al. 1991, Classen-Bockhoff 2016
Bruniacaeae	*Brunia*	Cape, S Africa	12/12/37	Globular, many-fruited raceme of dry nutlets (indehiscent) or woody capsules (dehiscent)	Wingless seeds or single-seeded nutlets	Sclerophyll low heath to scrub-heath	?	?	Lamont et al. 1991, Classen-Bockhoff 2016
Bruniacaeae	*Audouinia*	Cape, S Africa	1/5?/5	Subglobular, few-fruited raceme of sclerified capsules that dehisce laterally to release seeds after fire	Wingless seeds (dehiscent)	Sclerophyll low heath to scrub-heath	Yes	Sclerophyll low heath to scrub-heath	Classen-Bockhoff 2016
Bruniacaeae	*Staavia*	Cape, S Africa	3/3/11	Solitary, smooth, semiglobose, woody capsules (dehiscent)	Wingless seeds, arillate	Sclerophyll low heath to scrub-heath	?	Sclerophyll low heath to scrub-heath	Classen-Bockhoff 2016, B. Lamont observations from web images
Bruniacaeae	*Thamnia*	Cape, S Africa	1/1/9	Solitary, smooth, semiglobose, woody capsules (dehiscent)	Wingless seeds	Sclerophyll low heath to scrub-heath	?	Sclerophyll low heath to scrub-heath	Classen-Bockhoff 2016, B. Lamont observations from web images
Ericaceae	*Erica*	Africa and Europe	1/859?/860	*E. sessiliflora*: subglobular, many-fruited spike of dry nutlets with red, fleshy sepals to form a compound structure of berry-like fruits at intervals along stem	Single-seeded dry nutlets (indehiscent)	Sclerophyll low heath to scrub-heath	Yes	Sclerophyll low heath to forest in Europe	Oliver and Oliver 2002

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Table S2. Typical values used and results obtained in a model to show the effect of serotiny on number of seeds stored per plant and gene support for serotiny available postfire over 10 y since reaching maturity. Note the progressive build up of the gene support and seeds stored with increasing retention of seeds with time. Thus, if some seeds were held for 6 y, the total gene support would be 232 units (50 for the current crop) and seed store 413 (100 for current crop) with a mean gene support for serotiny (S_6) of 0.605 per seed (0.500 initial crop).

Year	Seeds produced	Fraction of seeds retained	Viability of seeds retained	Progressive total gene support G ($S_1 = 0.5$)	Progressive seed store H	Mean gene support per seed S_x (G/H)
1 (current)	100	1.00	1.00	50	100	0.500
2	95	0.95	0.95	95	186	0.512
3	90	0.90	0.90	136	259	0.524
4	85	0.85	0.85	172	320	0.537
5	80	0.80	0.80	204	371	0.549
6	75	0.75	0.75	232	413	0.561
7	70	0.70	0.70	256	448	0.573
8	65	0.65	0.65	278	475	0.584
9	60	0.60	0.60	296	497	0.595
10	55	0.55	0.55	311	513	0.605

Table S3. Number of new lineages/species per 5-million-year intervals that are either serotinous (S) or nonserotinous (N) for *Pinus* (Pinaceae – Northern Hemisphere), Callitroideae (Cupressaceae – Southern Hemisphere), *Protea* (Proteaceae – Africa) and *Hakea* (Proteaceae – Australia) based on Bayesian ancestral trait reconstruction techniques (Lamont, He and Yan 2019a). 0 to the left of all other variables means lineages with that trait yet to evolve in that interval while 0 among other values means there was no trait proliferation in that interval. – means the clade did not exist at that time. S means rate of proliferation of S > N, N means rate of proliferation of N > S, = mean rates are the same. Values within the table in bold are the highest rate recorded for that trait and clade. When a 10-My interval from a previous analysis was converted to a 5-My interval with the margins at X and (X + 1) this was given as the mean, (X + 0.5).

		Million years ago at 5-million-y intervals												
Clade	**Trait**	**60-55**	**55-50**	**50-45**	**45-40**	**40-35**	**35-30**	**30-25**	**25-20**	**20-15**	**15-10**	**10-5**	**5-0**	**Source**
Pinus	Serotinous	0	1	3	3	3	3	4	7	10	16	20	**24**	Fig. 10
	Nonserotinous	1	3	6	6	9	13	17	22	33	43	66	**91**	Fig. 10
		N	N	N	N	N	N	N	N	N	N	N	N	
Callitr	Serotinous	0.5	1	1.5	2	1	0	4	**6**	4	3	4	5	Fig. 12
oideae	Nonserotinous	0	0	1	2	1.5	1	0.5	0	1.5	2	5	**8**	Fig. 12
		S	S	S	=	N	N	S	S	S	S	N	N	
Protea	Serotinous	–	–	–	–	–	–	1	1	3	11	36	**70**	Fig. 10
	Nonserotinous	–	–	–	–	–	–	0	0	0	1	6	**17**	Fig. 10
		–	–	–	–	–	–	S	S	S	S	S	S	
Hakea	Serotinous - strong	–	–	–	–	–	–	–	1	2	**18**	15	4	Fig. 11
	Serotinous - weak	–	–	–	–	–	–	–	0	0	0	**4**	0	Fig. 11*
	Nonserotinous	–	–	–	–	–	–	–	0	0	1	**4**	1	Fig. 11*
		–	–	–	–	–	–	–	S	S	S	S	S	
Global	Serotinous	–	–	–	–	–	–	–	15	19	48	75	**103**	As above
	Nonserotinous	–	–	–	–	–	–	–	22	34.5	47	81	**117**	As above
		–	–	–	–	–	–	–	N	N	S	N	N	

* plus Lamont et al. (2017b)

Figures

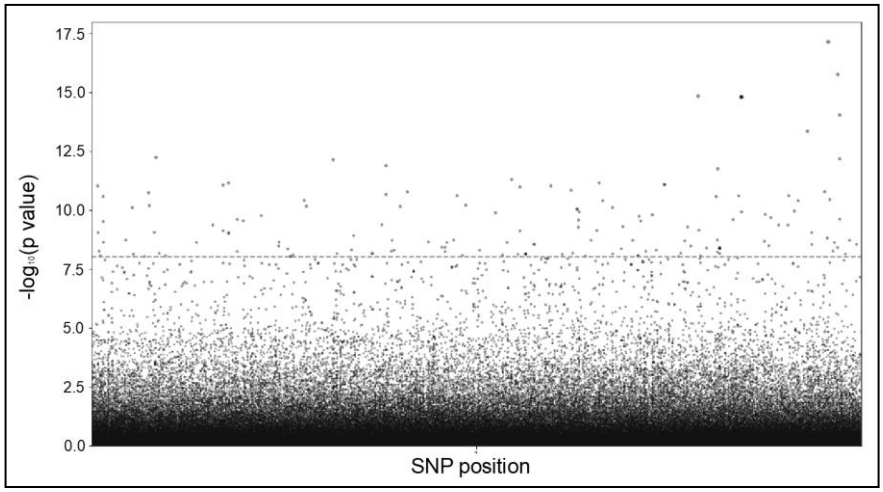

Fig. S1. Genome-wide association study showing the large number of single nucleotide polymorphisms (SNPs) associated with the level of serotiny in *Banksia attenuata*. The dotted line indicates the *P*-value threshold ($P = 2.62 \times 10^{-8}$, equivalent to $P = 0.010$ with Bonferroni correction for multiple tests). Leaves of this species were collected at nine locations along a rainfall gradient from the semi-arid region of Kalbarri to the high-rainfall *Cape Naturaliste* of SW Australia. He et al. (2016b, 2019) generated a genome-wide (SNP) profile. We determined the level of serotiny at each location by calculating the percentage of closed follicles per cone for two representative one-y-old cones from five plants per location. The level of serotiny varied from non- to weak serotiny (0–5% closed follicles) at the mesic sites with low frequency of crown fires to strong serotiny (>90% closed follicles) at the xeric sites with moderately frequent crown fires, consistent with a shorter gradient assessed by Cowling and Lamont (1985a) who used the slope measure of serotiny. Following a linear mixed model implemented in the software FaST-LMM (Lippert et al., 2011), a genome-wide association study analysis was used to determine what SNPs are associated with the level of serotiny.